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The Effect of Calcium and Magnesium Levels on the in Vitro Growth of Sterile Potato Cuttings

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THE EFFECT OF CALCIUM AND MAGNESIUM LEVELS ON THE *IN VITRO* GROWTH OF STERILE POTATO CUTTINGS.

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Of the Requirements for the Degree of

Master of Arts

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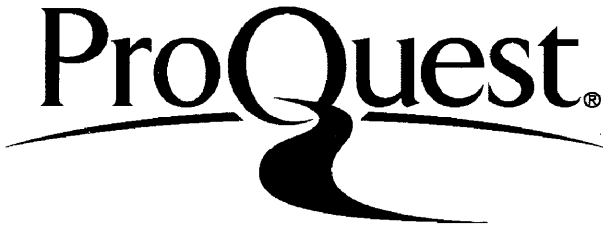
by

John Edward Chapman, Jr.

1988



APPROVAL SHEET

This thesis is submitted in partial fulfillment of

the requirements for the degree of

Master of Arts

J. Edward Chapman Jr.
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Approved, December 12, 1988.

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ABSTRACT

The vegetative propagation of potatoes using sterile stem cuttings has been shown to be limited by the occurrence of abnormal growth, namely shoot-tip necrosis, resulting in many-branched, vitrified and or stunted plantlets. Shoot-tip necrosis has been linked to calcium concentration in the growth medium. Through manipulation of growth medium formulation, varietal responses to calcium and magnesium levels were explored. 'Monona', 'Norland', and 'Russet Burbank' were shown to grow well at at standard (3mM) calcium levels while 'Atlantic', a white-flowered variant of 'Atlantic', 'Belrus', 'Kennebec', and 'Superior' required elevated levels. Magnesium levels were investigated under conditions of limiting calcium. No decrease in the occurrence of tip necrosis was noted under a series of combined low magnesium, low calcium conditions. Supplemental levels of calcium ions are suggested as medium components in the formulation of culture media which increased the number of useable cuttings.

THE EFFECT OF CALCIUM AND MAGNESIUM LEVELS ON THE *IN VITRO* GROWTH OF STERILE POTATO CUTTINGS.

INTRODUCTION

Plant tissue culture has proven to be a valuable research tool in the quest for understanding the basic nutritional requirements of many species. A sterile, soil-less environment, carefully formulated growth media, and the use of genetically identical clone sections all contribute to a more controlled and easily manipulated experimental system that produces data with a reduced number of the variations that plagued more traditional plant growth techniques. The term 'tissue culture' will be used to describe the method of propagation. However, the actual experimentation was carried out on plantlets derived from stem cuttings originally obtained from clone lines derived from apical meristem sections grown in vitro for over a year.

An understanding of the roles of the mineral elements, including calcium, in plant growth and metabolism has long been the goal of plant physiologists. Calcium was recognized as an essential element in higher plants during the middle of the last century, although the use of limestone and marl in agriculture dates back to pre-Christian times (Jones and Lunt 1985).

The free calcium ion is no longer regarded as a simple macronutrient in plants, but rather as a major intracellular regulator of metabolism and development. Calcium appears to function as a second messenger, transducing primary stimuli into physiological responses (Owen and Napier 1988). The function of calcium as a second messenger in animal cells has been acknowledged for years, but it is only recently that physiologists have come to appreciate the concept that activities of plant cells are regulated by calcium (Hepler and Wayne 1985). 'Second messenger' refers to the hypothesis describing the stimulation or inhibition of enzymes within a target cell by an extracellular first messenger unable to penetrate the cell membrane. The actual stimulation or inhibition is elicited by an intracellular second messenger produced in response to the first messenger (Eckert and Randall 1978). Cyclic adenosine monophoshate, or cAMP, at first was proposed to be the second messenger for all hormonal responses, and calcium ions the second messenger in muscle contraction, secretion, and egg activation (Hepler and Wayne 1985). Through continued experimentation it became apparent that the participation of calcium ions was more widespread than had been thought originally (Hepler and Wayne 1985). A universal calcium ion

messenger system emerged with cAMP as an additional system built, in part, upon its interaction with calcium ions (Rasmussen 1970, 1981, as cited by Hepler and Wayne 1985). Since cAMP-dependent protein kinase, the only known physiologically important receptor of cAMP, has never been found in plant systems, and cAMP itself has never been shown to be required for any physiological response in plants, calcium ions alone may contribute to the coupling of stimulus to response (Hepler and Wayne 1985).

The general mechanism by which calcium modulates a response is through concentration changes mediated by the modulator protein calmodulin (Hepler and Wayne 1985). In most cases, an extracellular stimulus temporarily increases calcium concentration by altering calcium channel activity at the plasmalemma. This elevated cytosolic calcium concentration can be sensed by the calcium-binding protein calmodulin and the resulting calcium-calmodulin complex then modulates enzyme activity, thereby elicitng a physiological response (Owen and Napier 1988).

The incorporation of calcium, in the form of calcium pectate, as a critical element in cell wall formation is perhaps the most frequently cited role of this element. The hypothesis of calcium pectate acting

as a cementing agent in the cell wall of plant cells was originated during the last century (True 1922) and has found general acceptance in plant physiology texts (Jones and Lunt 1985). Considerable evidence has accumulated that the formation of calcium pectate increases the rigidity of the cell wall (Tagawa and Bonner 1957, Rasmussen 1966, Cormack 1965, as cited by Jones and Lunt 1985). A more complex relationship between cell rigidity, elongation, and calcium is indicated by studies of Burstrom (1952, 1954, 1957). He concluded that root cell growth occurred in two stages: a) an increase in plasticity and elasticity of the cell wall, and b) the biosynthesis and laying down of new cell wall material (Jones and Lunt 1985). The first stage is enhanced by auxin but antagonized by calcium, whereas the relationship is reversed in the second stage (Jones and Lunt 1985). It is difficult to determine whether these interactions are related to a direct influence on the cell wall or to a more subtle alteration in metabolism through calcium's interaction with membranes (Jones and Lunt 1985).

Studies on the submicroscopic aspects of mineral deficiency, namely calcium, suggest that calcium is essential for the maintenance and probably the formation of cell membrane systems on which the

functional integrity of cell metabolism is dependent. The first indisputable signs of structural abnormalities appear when the nuclear envelope and the plasma and vacuolar membranes break up and structureless areas appear in the cells, followed by the disorganization of other structures such as mitochondria and Golgi apparatus (Marinos 1962). With the progress of calcium deficiency the cell walls stain darker and gaps may appear, indicating a weakening of their structure. Calcium effects on the cell walls are probably secondary to those already described (Marinos 1962). The role of calcium in the the ultrastructural alteration of plant membranes was shown to occur in isolated and in situ plasma membranes treated with calcium chloride, which were found to increase in thickness 15 to 20 percent (Morre and Bracker 1976).

The effects of calcium extend beyond the cell wall to embrace disparate areas of plant growth and development. Calcium is implicated in the orientation of polarized growth in developing zygotes. By carrying part of the transcellular electrical current, calcium is thought to mediate bioelectric potentials. Studies of tip growing plant cells such as pollen tubes, root hairs, and moss protonemata show that a tip-to-base calcium ion gradient exists, the results interpreted as

evidence for a general role of a calcium gradient in tip growth (Reiss and Herth 1979). Changes in the structure of the tips of pollen tubes after transfer to inhibitory calcium ion conditions provide evidence in support of a mechanism of pollen tube tip growth. Tip extension is controlled, not only by the rate of vesicle fusion, but also by the state of plasticity of the tip; both of these processes appear to be sensitive to changes in calcium ion concentration (Picton and Steer 1983). It is widely assumed that calcium ions help regulate mitosis and cytokinesis through the formation of cell plates and mitotic apparatus (Hepler and Wayne 1985). The presence of calcium is necessary for mitochondrial uptake and concentration of phosphate, magnesium, and calcium (Hodges and Hanson 1965). At the root level, calcium ions are essential for the integrity of the selective absorption mechanism responsible for mantaining proper potassium and sodium concentrations (Epstein 1961). The time-dependent stimulation of transport rate, maintenance of high transport rates, and the retention of transported serine, are known to be calcium dependent (Smith 1978). Calcium has also been shown to affect aging in plant leaf tissue. Senescence of corn leaf discs was deferred by added calcium, the effect

being additive to deferral caused by cytokinin. Deferral of senescence with added calcium was also observed in Rumex obtusifolius L. leaf disc (Poovaiah and Leopold 1973).

Calcium also influences development by affecting the production and transport of certain plant hormones. Cytokinin-stimulated ethylene production, in studies using cotyledon expansion assay, is now understood to be related to the relative concentrations of calcium and potassium, but not directly to the presence of either ion (Green 1983). Studies of the effect of kinetin on calcium uptake and of calcium on the uptake and metabolism of kinetin in relation to their effect on ethylene production show that application of kinetin and calcium ion caused a striking synergistic increase in ethylene production by mung bean hypocotyl segments (Lau and Yang 1975). Studies using sunflower hypocotyls suggest that the rate of auxin transport in plant tissue is dependent on the pool of ionic calcium in the extracellular space. Other divalent cations tested for their ability to replace calcium in restoring auxin transport showed no effect (DeLa Fuente 1984). The calcium effects are interpreted as indicating that the auxin transport system depends upon structural or functional features of cellular membranes

which involve calcium in a manner analogous to the transport of inorganic ions (DeLa Fuente and Leopold 1973). It has been shown that abnormal tissue development, particularly the vitrification of leaves, is related to impaired polarity and reduction of auxin transport (Werker and Leshem 1987, Gersani et al. 1986).

The visual effects of calcium deficiency fall into three principal symptoms. Low calcium availability may result in blackening and curling of the margins of the apical leaves, leading to necrosis and cessation of growth. The physiological foundation for this deformation may be complex, however, as it is thought to be closely related to the magnesium status, because it occurs more readily at high magnesium levels (Hewitt 1963; E. Frolich, unpublished observations 1966, as cited by Jones and Lunt 1985). Poor root development, along with reduced fruit and seed formation, are also evident under low calcium conditions (Jones and Lunt 1985). Calcium's influence is not clear: however, it may be that there is a calcium requirement for membrane integrity which may be more acute in dividing cells. Since calcium is poorly translocated (Biddulph et al. 1959), it would also be expected that the apex would be more severely affected

(Jones and Lunt 1985). Calcium, which typically exists as a divalent cation in aqueous solution, is not subject to a significant redistribution within the plant, but typically remains stationary in the place to which it was delivered.

Calcium is carried through the plant system in two ways. The majority of calcium transport is accomplished along with the mass movement of water in the vessels, while a significant portion of the ascending calcium moves by exchange on biocolloids within the stem as a whole (Biddulph et al. 1961). Studies with strawberry plants indicate that calcium transport to newly emerged, non-transpiring leaves is dependent on the water flow rising from root pressure at night. After leaf emergence, calcium intake into leaves is then supplied by transpirational water flow (Bradfield and Guttridge 1979). Once in place, there is very little redistribution of calcium ions within the plant tissues. Movement of calcium ions at the cellular level is accomplished via specific channels structured to handle calcium ions and no other, in much the same way as sodium, potassium, and other ions are handled by individual regulatory channels governed by specific proteins (Owen and Napier 1988).

The Solanum tuberosum system is particularly

suited for a mineral nutrition study. Owing to its importance as an agricultural species, and its relatedness to other well-studied species, the potato's habits and requirements are comparatively well understood *in vitro* (Hussey and Stacey 1981).

In 1985 Liu Sha *et al*. published data concerning the occurrence and cause of shoot-tip necrosis in sterile shoot cultures of *Solanum tuberosum*. Using three cultivars grown on media of varying calcium concentration they observed reduced occurrence rates of necrosis with added calcium and increased rates with reduced calcium. The incidence of abnormal growth in tissue cultured *S. tuberosum* was further explored in the present study.

MATERIALS AND METHODS

Stock clone lines were obtained from actively growing stock cultures maintained for at least one year *in vitro*. These cultures originated at the Maine Seed Potato Board's tissue culture facility at the Porter Farm in Masardis, Maine. This facility specializes in the production of disease-free seed potato progenitors through tissue culture multiplication techniques and also acts as a repository for many of the potato varieties grown in the United States.

The varieties used were 'Atlantic', 'WF31-4', 'Belrus', 'Kennebec', 'Monona', 'Norland', 'Russet Burbank', and 'Superior'. 'Russet Burbank', 'Superior', and 'Norland' were the three cultivars used by Sha *et al*. (1985) and were included for comparison. The remaining five varieties were chosen after consultation with David Hammond, director of the Porter Farm facility, to better represent a full spectrum of symptomatic responses to mineral deficiencies. 'Belrus' is strongly determinate in growth habit, has a smaller root system, and is noticeably less vigorous

than average. 'Atlantic' is noted for its rapid growth and development and large tuber set count. It is also less susceptible to heat lesions and necrosis, a possible calcium concentration related attribute. 'WF31-4' is a white-flowered selection of 'Atlantic', which is lavender-flowered. 'Kennebec' is a vigorous and large grower, a popular and long-standing variety in commercial potato farming. 'Monona' has demonstrated a strong link between plant vigour/yield and calcium levels in the soil at the Porter Farm. Each varietal progenitor clone was tested for the presence of potato pathogens using ELISA and other protocols. All tested negative for the specific viruses X, Y, potato mosaic virus, potato leaf roll virus, spindle tuber retrovirus, and the blackleg bacteria.

Three major changes were made in the design of the experiments discussed in this paper as compared to the work performed by Sha et al. (1985). First, the number of cultivars used was expanded from three to eight to better represent the diversity of varietal responses to different calcium levels. Second, the media concentrations of magnesium were varied under low calcium conditions to explore the suggested calcium - magnesium relationship. Third, varietal responses to

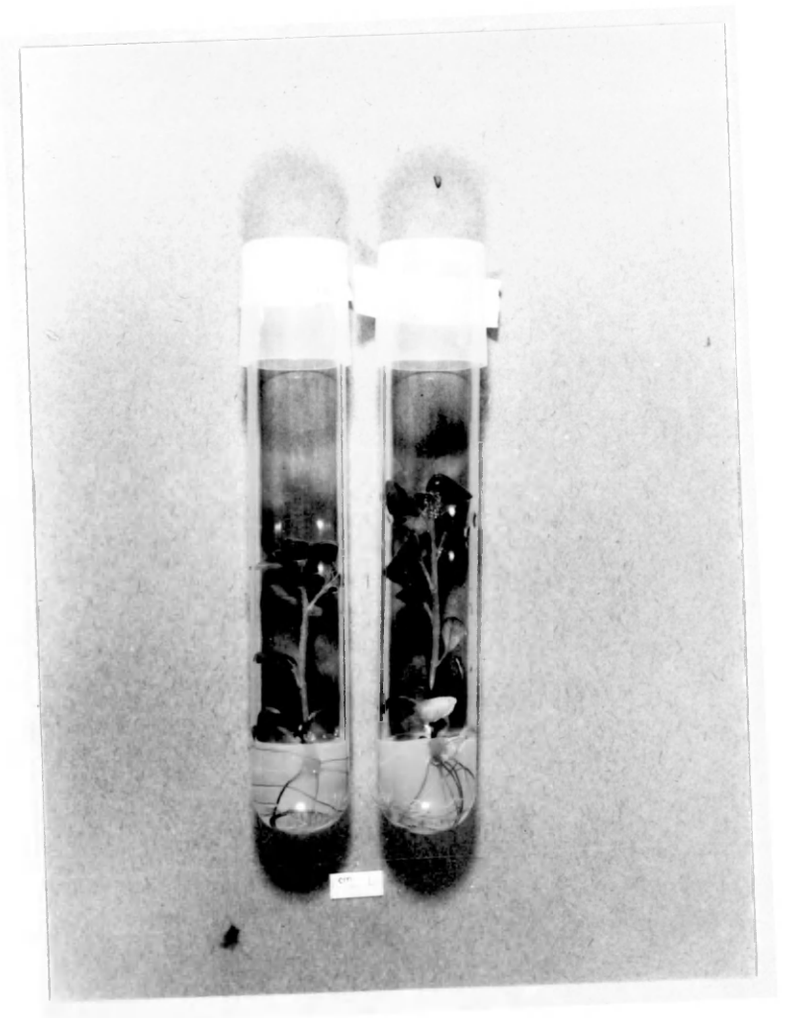
specific calcium and magnesium levels were broken down into different symptoms, including tip necrosis.

One sterile shoot section consisting of one node complete with leaf, axillary bud, and at least one centimeter of stem was placed in a sterile 125X50 borosilicate culture tube containing ten milliliters of agar growth medium (Figure 1) The nodal sections were inserted upright in the medium so as to place the leaf base in contact with the agar surface. Careful use of the stem tissue above the axillary bud as a 'handle' insured that the leaf and bud were not damaged in the process of manipulation and placement. Each tube was then capped with a "Magenta Two-Way Cap" and placed in a culture room maintained at 25-30 C. Lighting was supplied by banks of flourescent lights at 800-1000 lux. Photoperiod was set at sixteen hours light and eight hours darkness.

Sha et al. (1985) used 220 ml baby food jars, each containing five stem cuttings and 30ml of media. My preliminary experimentation with similar containers and multiple cuttings per container yielded uneven growth and poor development. This led to the decision to use the single cutting culture tube, as the individual plantlets grew more evenly in respect to each other, were more easily observed, and could not

Figure 1.

Culture tube set-up with 15 day old plantlets.



affect the growth of each other directly. This set-up is the one of choice used at the Maine facility mainly because of its high yield of suitable cuttings, and ease of manipulation. Sha et al. (1985) chose to place the cutting horizontally on the surface of the medium. My experimentation with various techniques led to the decision to insert the cutting into the medium, as it yielded the most vigorous and even growth. Since much of the data gathered in this paper relies on differences in growth, any reduction in variability due to factors other than mineral concentrations and variety will clarify the results.

At the end of thirty days growth the height of each plantlet and the number of nodes were recorded along with observations of general appearance, root growth, color, etc. Height was measured to plus/minus two millimeters, starting from the agar surface to the apical meristem, with maximum height determined by the limits of the tube at 125 mm. Visible nodes were counted up to the apical tip to include all those bearing a leaf and elongated internode.

Part one of this study explores the effects of low calcium levels on shoot growth and development by altering the calcium concentration in the growth medium consistent with Sha et al. (1985). Experimentation was

carried out using equipment and materials provided by the Maine facility. Murashige and Skoog revised medium was used throughout and modified to give three calcium concentration levels (Table 1) (Murashige and Skoog 1962). Agar was used at the rate of six grams per liter and pH was adjusted to 5.7. Twelve tubes of each potato variety in each of the three calcium concentrations were prepared. Three replications were run for each, giving a total of thirty-six tubes per variety per treatment. In the case of 'WF31-4' only eight tubes per variety were available, giving a total of twenty-four tubes.

Part two of the study explores the effect of magnesium concentration on shoot development under the 'low' calcium concentration used in part one. This segment of experimentation was performed using equipment provided by the College of William and Mary Biology Department, and identical clone stocks brought back from the Maine facility. The magnesium level of the medium was manipulated by increasing the concentration of magnesium sulfate by factors of ten while holding the calcium level constant (Table 1). This yielded three experimental medium formulations designated 'Standard' with magnesium at 1.5 mM and calcium at 0.3 mM, 'Low" with magnesium at 0.15 mM and

TABLE 1

Nutrient Sources

Nutrient	Source	Concentration in medium (mM) Low Ca	Standard Ca	High Ca
Ca	CaCl2	0.3	3	---
	Ca(NO3)2	---	---	30
Cl	CaCl2	0.6	6	---
	NH4Cl	---	---	6
	KCl	---	---	10
NO3	Ca(NO3)2	---	---	60
	NH4NO3	20	20	---
	KNO3	20	20	---
NH4	NH4NO3	20	20	---
	NH4Cl	---	---	6
K	KNO3	20	20	---
	KCl	---	---	10
	K2SO4	---	---	8
SO4	K2SO4	---	---	4
MG	MGSO4	1.5	1.5	1.5
	multiple	1.7	1.7	1.7

(Sha 1985)

calcium at 0.3 mM, and 'Trace' with magnesium at 0.015 mM and calcium at 0.3 mM. Sodium sulfate was added to low and trace media to compensate for loss of sulfate ion. Set-up and growth of individual tubes were identical to part one, with the exception that one replication of seven tubes for each variety, under each of the three treatments, was used due to space constraints. A temperature discrepency was also noted between the two experiments. The Maine facility growth room mantained a temperature ranging from 25 - 30 C., with an average temperature toward the high end at about 27 degrees. The controlled environment chamber at William and Mary maintained a range of temperature between 23 and 28 degrees, with an average temperature toward the lower end at about 24 degrees. Each of the eight varieties were grown under conditions of standard (1.5 mM), low (0.15 mM) and trace (0.015 mM) magnesium levels, with the calcium level set at 0.3 mM for all treatments. In this experiment abnormal growth was broken down into five characterizations, and the appearance of each, either alone or in combination with others, was noted for each cutting. Total plantlet height, number of nodes, and number of lateral shoots were also noted for each plantlet.

The effects of calcium concentration in the

medium on the growth of sterile stem cuttings *in vitro* were assayed using three parameters. Each cutting was recorded as either abnormal or normal in terms of its growth pattern. Normal growth is defined as a single-stemmed cutting with nodes evenly spaced and leaves healthy in appearance and size. Any deviation from this, whether it be tip necrosis only or some combination of necrosis, withering, and side-branching, constitutes an abnormal cutting in this phase of the experiment. Each cutting's height was also recorded, the measurement beginning at the medium surface and running to the tip of the primary shoot. In the case of many-branched cuttings, where the primary shoot could not be discerned, the height of the tallest branch was used. The number of nodes per cutting was the final growth parameter. A two-way analysis of variance was carried out for each of the two continuous variables: height and node number.

RESULTS AND DISCUSSION

In respect to height (Table 2), the means were significantly different between varieties and calcium levels. A significant interaction between calcium level and variety was also found. The results of a two-way ANOVA using the node number (Table 3) data yielded similar results: significant differences appeared in all combinations.

Abnormal growth occurred differentially among varieties and calcium concentrations (Table 4). Each of the eight selected varieties exhibited abnormal growth under the low calcium treatment. Of these, 'Belrus', 'Kennebec', 'Russet Burbank', and 'Superior' were the most sensitive with one hundred percent of the plantlets showing abnormality to some degree (Fig 2-10). Previous research using the cultivars 'Russett Burbank', 'Superior', and 'Norland' produced similar results under the same concentration. Sha *et al.* (1985) found 'Russett Burbank' to be the most sensitive of the three tested varieties, showing a 72 percent tip necrosis rate. 'Superior' followed with 62 percent,

TABLE 2

MEAN HEIGHT* OF PLANTLETS GROWN FOR THIRTY DAYS UNDER THREE LEVELS OF CALCIUM CONCENTRATION

CALCIUM LOW	MEAN	STD DEV	N
ATLANTIC	54.306	12.359	36
WF31-4	68.958	18.088	24
BELRUS	49.139	15.104	36
KENNEBEC	45.914	9.696	35
MONONA	50.306	9.155	36
NORLAND	67.694	10.786	36
RUSSETB	57.750	10.377	36
SUPERIOR	62.861	15.956	36
GRAND MEAN	57.116		
CALCIUM STANDARD			
ATLANTIC	61.273	7.954	33
WF31-4	79.125	16.459	24
BELRUS	104.972	21.614	36
KENNEBEC	79.444	19.504	36
MONONA	56.167	14.147	36
NORLAND	79.556	13.464	36
RUSSETB	98.686	17.070	35
SUPERIOR	101.556	17.443	36
GRAND MEAN	82.597		
CALCIUM HIGH			
ATLANTIC	67.235	14.039	34
WF31-4	88.000	21.921	24
BELRUS	108.194	18.522	36
KENNEBEC	86.472	17.354	36
MONONA	45.600	10.036	35
NORLAND	81.167	17.472	36
RUSSETB	101.833	15.760	36
SUPERIOR	97.667	16.306	36
GRAND MEAN	84.521		
FOR ENTIRE SAMPLE	74.680	25.254	820

TABLE 3

MEAN NUMBER OF NODES* PER PLANTLET GROWN FOR THIRTY DAYS UNDER THREE LEVELS OF CALCIUM CONCENTRATION

CALCIUM LOW	MEAN	STD DEV	N
ATLANTIC	5.306	1.390	36
WF31-4	5.750	2.111	24
BELRUS	2.694	.525	36
KENNEBEC	3.400	.976	35
MONONA	7.028	1.464	36
NORLAND	6.556	1.157	36
RUSSETB	4.528	1.055	36
SUPERIOR	3.778	1.312	36
GRAND MEAN	4.880		
CALCIUM STANDARD			
ATLANTIC	6.364	.742	33
WF31-4	6.875	1.191	24
BELRUS	7.472	1.934	36
KENNEBEC	6.417	1.052	36
MONONA	6.639	.899	36
NORLAND	7.111	.887	36
RUSSETB	7.657	.802	35
SUPERIOR	6.306	.856	36
GRAND MEAN	6.855		
CALCIUM HIGH			
ATLANTIC	6.294	.970	34
WF31-4	6.875	.850	24
BELRUS	7.639	1.018	36
KENNEBEC	6.194	.822	36
MONONA	6.629	.646	35
NORLAND	6.833	.811	36
RUSSETB	6.833	.655	36
SUPERIOR	6.778	.832	36
GRAND MEAN	6.634		
FOR ENTIRE SAMPLE	6.150	1.693	820

TABLE 4

PERCENTAGE OF PLANTLETS SHOWING ABNORMAL GROWTH* AFTER THIRTY DAYS AT THREE LEVELS OF CALCIUM CONCENTRATION

VARIETY	LOW (0.3 mM)	STANDARD(3.0 mM)	HIGH(30.0 mM)
ATLANTIC	72.22	5.56	0
WF31-4	58.33	4.17	0
BELRUS	100.00	33.33	0
KENNEBEC	100.00	47.22	0
MONONA	66.67	0	0
NORLAND	58.33	0	5.56
RUSSET BURBANK	100.00	0	0
SUPERIOR	100.00	22.22	2.78
AVERAGE PERCENT	81.94	14.06	1.04

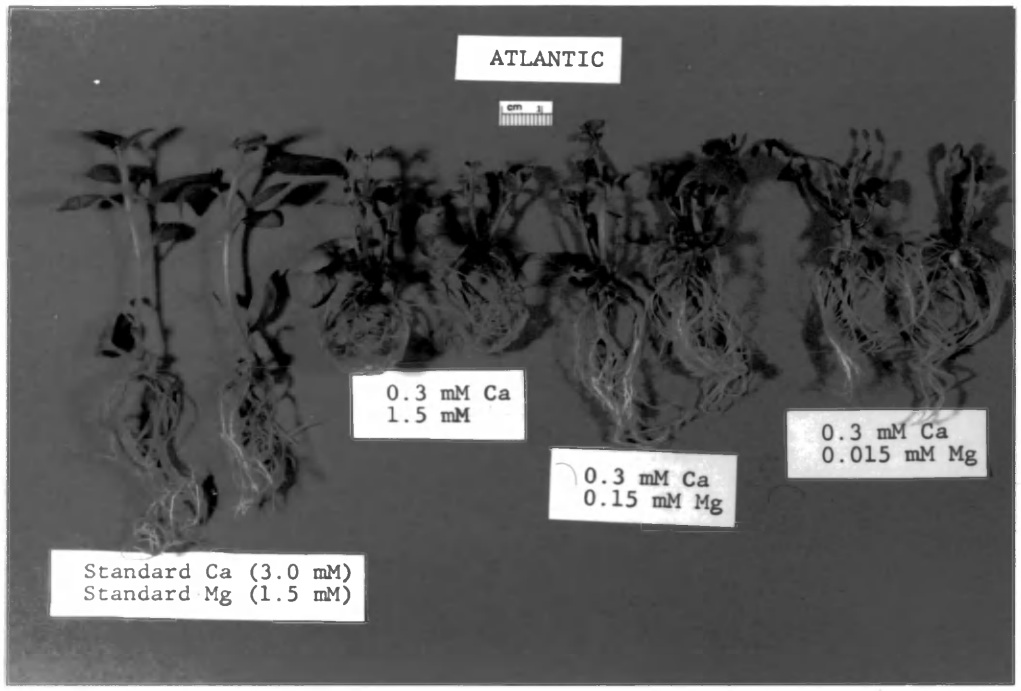
*Abnormal growth is defined as any change in plantlet form that would render it unsuitable for subdivision into usable cuttings. This includes tip necrosis, which may or may not be accompanied by release of lateral meristems.

Figure 2.

'Atlantic' grown for thirty days under low calcium /variable magnesium conditions.
Left to right:
Standard calcium/magnesium control
Low calcium/standard magnesium control
Low calcium/low magnesium
Low calcium/trace magnesium

Figure 3.

'WF31-4' grown for thirty days under low calcium/variable magnesium conditions.
Left to right same as in Fig. 2.


ATLANTIC
0.3 mM Ca
1.5 mM
0.3 mM Ca
0.15 mM Mg
0.3 mM Ca
0.015 mM Mg
Standard Ca (3.0 mM)
Standard Mg (1.5 mM)

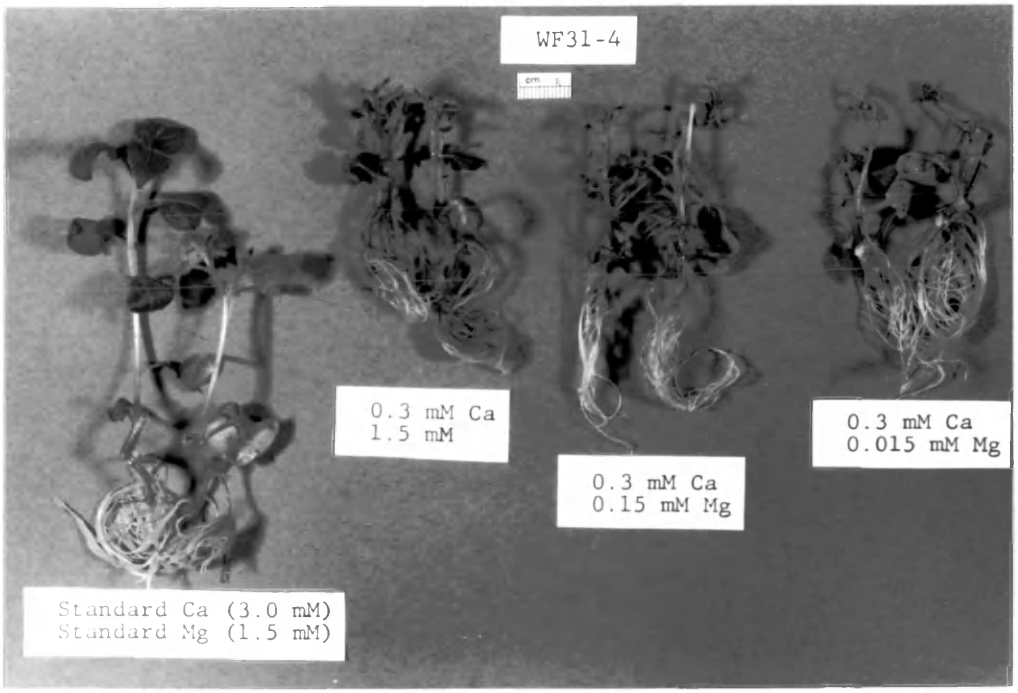

WF31-4
0.3 mM Ca
1.5 mM
0.3 mM Ca
0.15 mM Mg
0.3 mM Ca
0.015 mM Mg
Standard Ca (3.0 mM)
Standard Mg (1.5 mM)

Figure 4.

Comparison of low calcium/variable magnesium 'Atlantic' and 'WF31-4' plantlets.

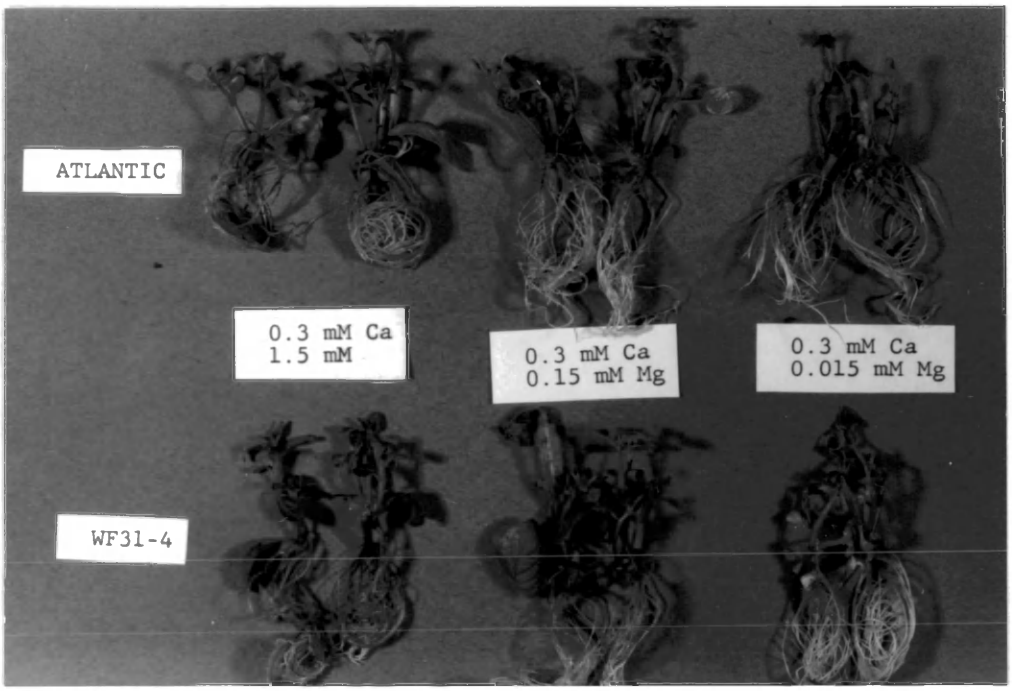
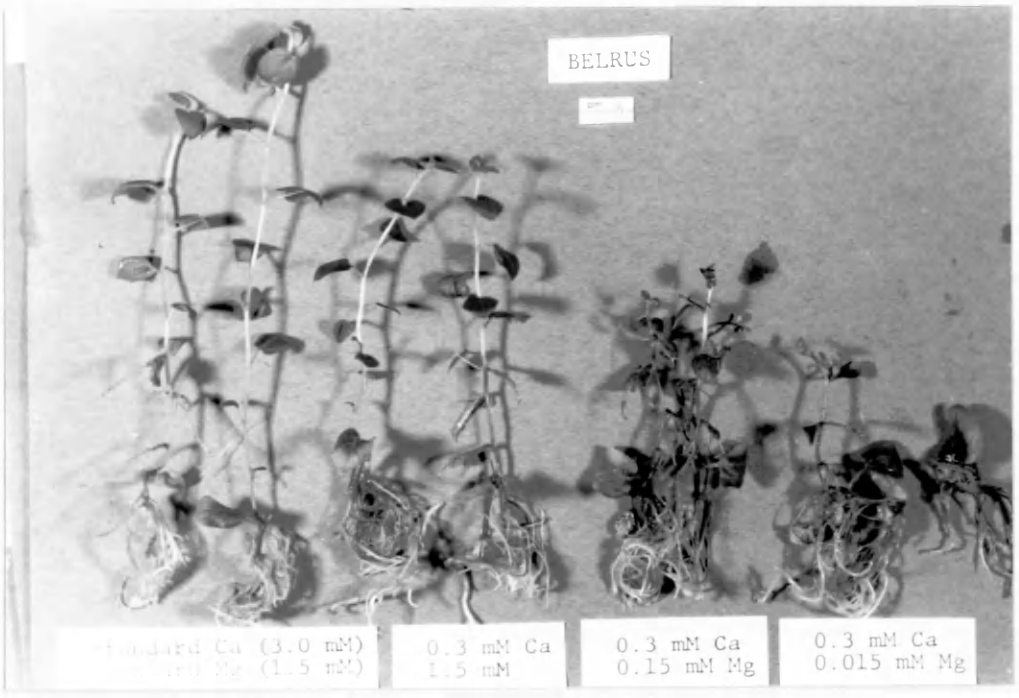

ATLANTIC
0.3 mM Ca
1.5 mM
0.3 mM Ca
0.15 mM Mg
0.3 mM Ca
0.015 mM Mg
WF31-4

Figure 5.

'Belrus' grown for thirty days under low calcium /variable magnesium conditions.
Left to right:
Standard calcium/magnesium control
Low calcium/standard magnesium control
Low calcium/low magnesium
Low calcium/trace magnesium

Figure 6.

'Monona' grown for thirty days under low calcium/variable magnesium conditions.
Left to right same as in Fig. 5.


BELRUS
Standard Ca (3.0 mM)
Standard Mg (1.5 mM)
0.3 mM Ca
1.5 mM
0.3 mM Ca
0.15 mM Mg
0.3 mM Ca
0.015 mM Mg

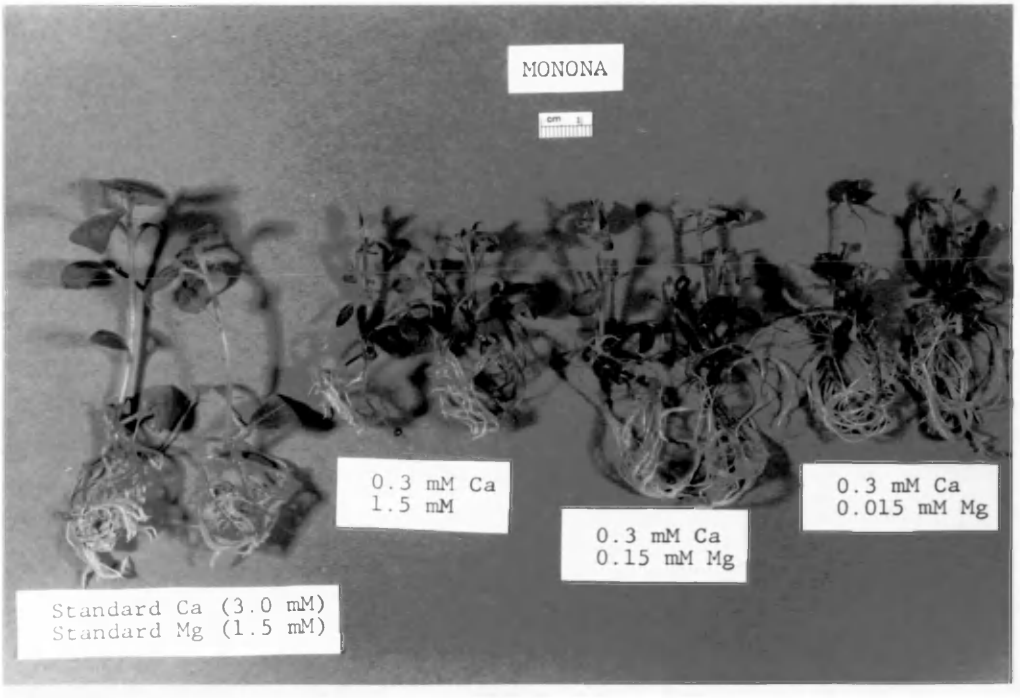
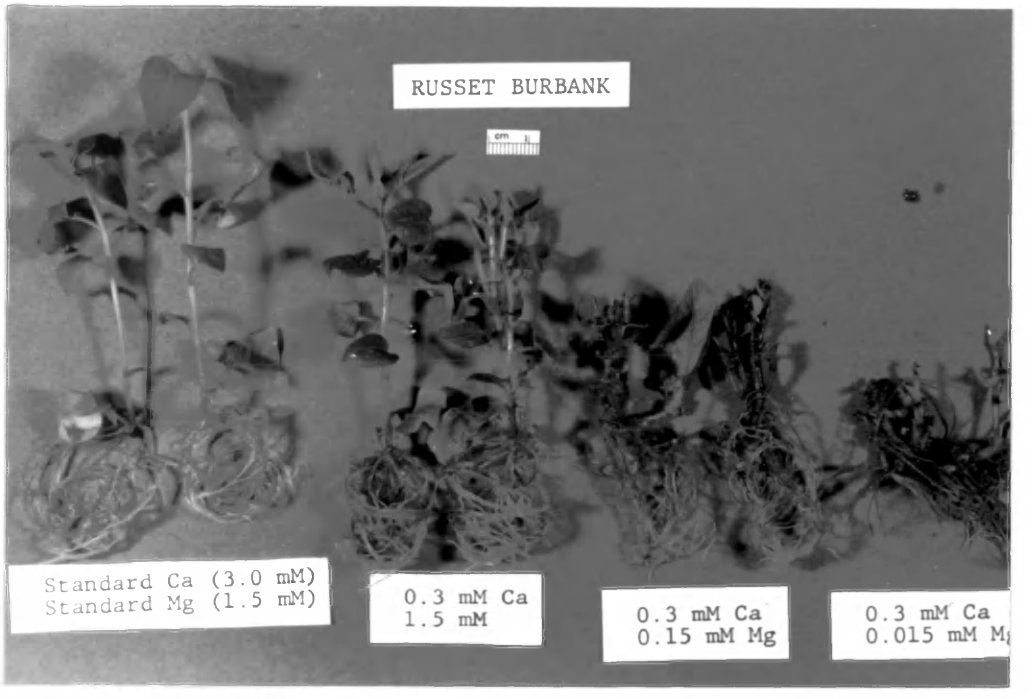

MONONA
0.3 mM Ca
1.5 mM
0.3 mM Ca
0.15 mM Mg
0.3 mM Ca
0.015 mM Mg
Standard Ca (3.0 mM)
Standard Mg (1.5 mM)

Figure 7.

'Russet Burbank' grown for thirty days under low calcium /variable magnesium conditions.
Left to right:
Standard calcium/magnesium control
Low calcium/standard magnesium control
Low calcium/low magnesium
Low calcium/trace magnesium

Figure 8.

'Superior' grown for thirty days under low calcium/variable magnesium conditions.
Left to right same as in Fig. 7.


RUSSET BURBANK
Standard Ca (3.0 mM)
Standard Mg (1.5 mM)
0.3 mM Ca
1.5 mM
0.3 mM Ca
0.15 mM Mg
0.3 mM Ca
0.015 mM Mg

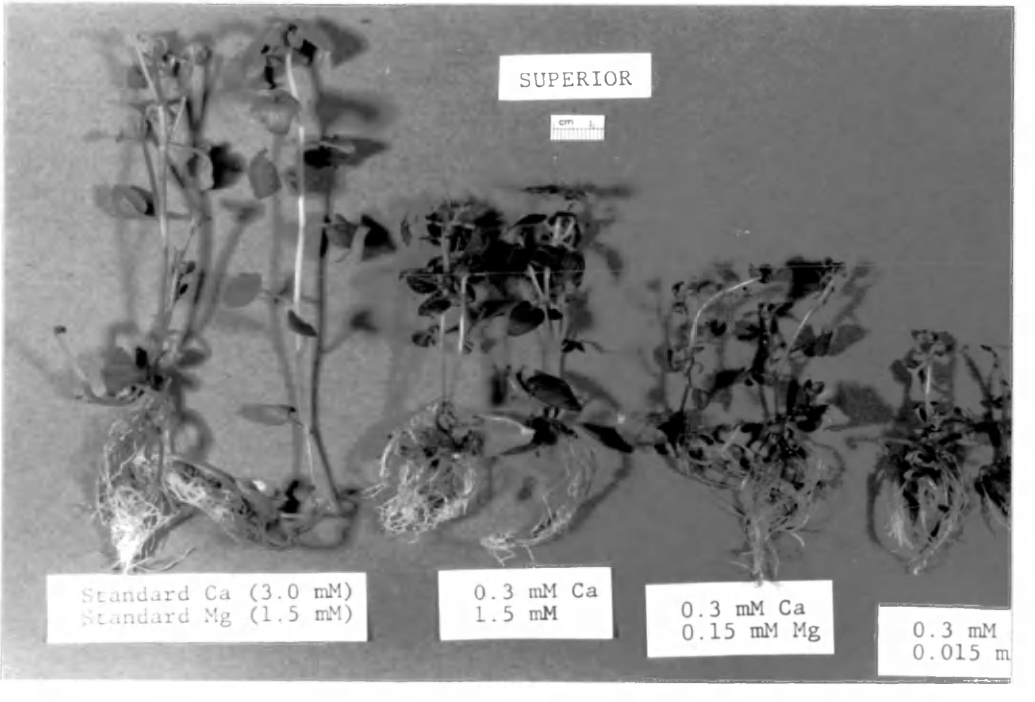
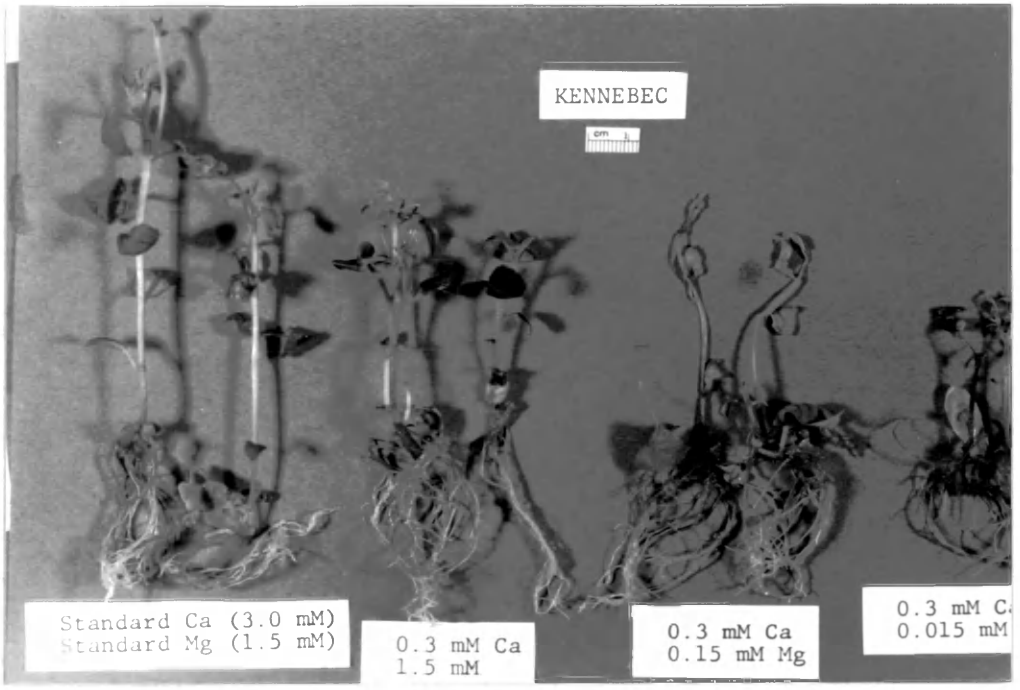

SUPERIOR
Standard Ca (3.0 mM)
Standard Mg (1.5 mM)
0.3 mM Ca
1.5 mM
0.3 mM Ca
0.15 mM Mg
0.3 mM
0.015 m

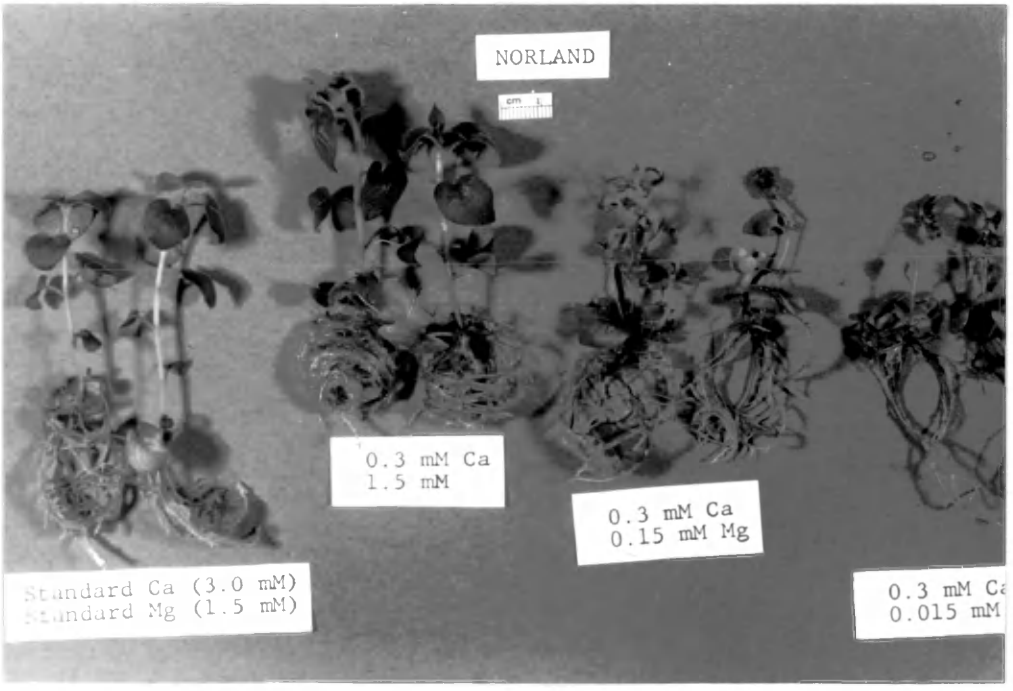
Figure 9.

'Kennebec' grown for thirty days under low calcium /variable magnesium conditions.
Left to right:
Standard calcium/magnesium control
Low calcium/standard magnesium control
Low calcium/low magnesium
Low calcium/trace magnesium

Figure 10.

'Norland' grown for thirty days under low calcium/variable magnesium conditions.
Left to right same as in fig. 9.


KENNEBEC
Standard Ca (3.0 mM)
Standard Mg (1.5 mM)
0.3 mM Ca
1.5 mM
0.3 mM Ca
0.15 mM Mg
0.3 mM Ca
0.015 mM


NORLAND
0.3 mM Ca
1.5 mM
0.3 mM Ca
0.15 mM Mg
Standard Ca (3.0 mM)
Standard Mg (1.5 mM)
0.3 mM Ca
0.015 mM

and 'Norland' last with 48 percent. 'Belrus', 'Kennebec', and 'Superior' continued to show abnormal growth under standard calcium concentration though to a lesser extent, while 'Russet Burbank' cleared up completely with all plantlets appearing normal. When compared to data from Sha et al. (1985) the results are similar, though the incidence of necrosis drops to under 10 per cent in all varieties grown at standard calcium levels. Under high calcium concentration nearly all abnormalities were alleviated in all varieties. 'Norland' behaved differently in that a significantly higher rate of abnormality occurred under high calcium than under standard calcium. Similar results were reported by Sha et al. (1985) where 'Norland' shows greater necrosis at the highest calcium level, rising from one percent necrosis at standard calcium level to three percent necrosis at high calcium level. It is noted by Sha et al. (1985) that there is no significant difference between the two percentages.

In respect to height, most of the varieties responded positively to increasing levels of calcium (Table 2). 'Atlantic', 'WF31-4', 'Belrus', 'Kennebec', 'Norland', and 'Russet Burbank' all showed the greatest increase in height under high calcium conditions. 'Monona' and 'Superior' however reached their maxima

under standard calcium conditions.

Node numbers under each of the three calcium treatments did not change as dramatically, although the differences were significant overall as shown by the ANOVA. Each variety produced the least number of nodes under low calcium conditions. However the change from standard to high calcium, in terms of node number, was not as great (Table 3). Therefore it is logical to assume that the increase in height observed under increasing levels of calcium in the medium is best explained by an increased elongation of the internode more than an increase in node production.

The manipulation of magnesium levels under low calcium concentration conditions in the medium, was used to explore the possible interaction of these two ions in the tissue culture system. The deformation caused by calcium deficiency is thought to be closely related to the magnesium status, and has been shown to occur most readily at high magnesium levels (Jones and Lunt 1985). The second experiment approaches this relationship from the opposite direction, namely subjecting calcium stressed plantlets to increasingly lower magnesium levels. If the incidence of abnormality decreases under lower magnesium levels, then it would be reasonable to assume that magnesium in

some ways becomes toxic in calcium stressed plants. This would indicate that it is the ion imbalance more so than the absence of calcium that elicits necrosis and/or other abnormal growth patterns.

Of the eight varieties tested, none showed a significant decrease in abnormal growth with a reduction in magnesium level (Table 5). Problems in growth associated with low calcium levels, e.g. tip necrosis, were more prevelent as magnesium levels decreased. Necrosis increased steadily from 16 to 82 to 91 percent, of the pooled varieties, as magnesium levels decreased. Callus formation followed a similar pattern, becoming more widespread. Interestingly, the formation of stoloniferous shoots, tubers, and aerial roots peaks under low magnesium, and decreases under trace magnesium. The high occurrence of each abnormality under low magnesium may be due to increased stress on the system, while the marked reduction under trace magnesium resulting from an overall decrease in growth due to deficiencies of magnesium and calcium.

In respect to height, node number, and lateral shoot number, a two-way ANOVA yielded significant differences in all cases. Each variable was analysed by variety (n=8) and magnesium level (n=3). 'Belrus', 'Kennebec', 'Monona', 'Norland', 'Russet Burbank',and

TABLE 5

NUMBER OF PLANTLETS SHOWING ABNORMAL GROWTH* AFTER THIRTY DAYS AT THREE LEVELS OF MAGNESIUM CONCENTRATION

VARIETY	STANDARD(1.5 mM)					LOW(0.15 mM)					TRACE(0.015 mM)				
	NEC	CAL	TUB	STO	AER	NEC	CAL	TUB	STO	AER	NEC	CAL	TUB	STO	AER
AT	0	0	0	0	0	6	6	0	1	4	6	6	0	0	7
WF	0	0	0	0	0	4	5	0	0	4	7	7	0	0	2
BR	0	0	0	7	6	3	0	7	7	7	4	2	5	7	6
KN	2	1	0	0	1	6	4	3	1	5	7	3	0	0	0
MN	6	6	0	0	0	7	7	0	1	1	7	7	0	1	1
NO	0	0	0	0	0	6	3	0	0	3	7	1	0	1	1
RB	0	0	0	0	0	7	7	0	0	6	7	5	0	0	1
SP	1	2	1	4	2	7	2	1	6	3	6	7	0	1	2
TOTAL	9	9	1	11	9	46	34	11	16	33	51	38	5	10	20
PERCENT (N=56)	16	16	2	20	16	82	61	20	29	59	91	68	9	18	36

*Abnormal growth is defined as any change in plantlet form that would render it unsuitable for subdivision into usable cuttings. This includes tip necrosis, which may or may not be accompanied by release of lateral meristems.
In this case abnormal growth is broken down into separate characteristics:

NEC= necrosis, noticeable death of stem tissue, usually occurring at the very tip, but often extending downward to include much of the stem.

CAL= callus, noticeable growth of callus tissue, usually on the roots, but often on the cutting basipetal end.

TUB= tuber, the growth of a tuber either on a stoloniferous lateral meristem growing down into the medium, or axillary above media surface.

STO= stolon, growth of axillary meristems into geotropic, lightly pigmented and leafless shoots that may or may not produce tubers.

AER= aerial roots, adventitious rooting, always above media and just below leaf juncture.

'Superior' showed decreasing height with decreasing magnesium concentration (Table 6). 'Atlantic' and the white-flowered selection 'WF31-4' behaved differently, with a slight increase in average plantlet height under low magnesium concentration. Similarly, each variety, with the exception of 'Norland', showed a progressive decrease in node number with decreasing magnesium concentration (Table 7). 'Norland' experienced slight increase in average node number per plantlet under trace magnesium levels. Lateral shoot counts in all varieties except 'Belrus' and 'Superior' peak under the low magnesium treatment (Table 8). The decrease in shoot number under trace magnesium in most varieties was probably due to overall decreased growth as shown by the height measurements.

TABLE 6

MEAN HEIGHT* OF PLANTLETS GROWN FOR THIRTY DAYS UNDER LOW CALCIUM AND THREE LEVELS OF MAGNESIUM

STANDARD MG(1.5 mM)	MEAN	STD DEV	N
ATLANTIC	32.714	5.499	7
WF31-4	41.286	16.398	7
BELRUS	94.571	29.866	7
KENNEBEC	53.714	8.789	7
MONONA	39.714	14.534	7
NORLAND	64.714	14.361	7
RUSSETB	62.429	10.706	7
SUPERIOR	54.714	15.489	7
GRAND MEAN	55.482		
LOW MG(0.15 mM)			
ATLANTIC	38.571	13.489	7
WF31-4	44.714	10.828	7
BELRUS	44.286	8.558	7
KENNEBEC	46.429	10.358	7
MONONA	36.429	7.277	7
NORLAND	30.857	13.297	7
RUSSETB	25.714	4.957	7
SUPERIOR	36.714	17.433	7
GRAND MEAN	37.964		
MG TRACE(0.015 mM)			
ATLANTIC	27.571	3.359	7
WF31-4	33.714	6.525	7
BELRUS	46.714	18.768	7
KENNEBEC	44.000	13.796	7
MONONA	21.714	6.047	7
NORLAND	44.143	7.734	7
RUSSETB	19.286	5.314	7
SUPERIOR	31.857	12.456	7
GRAND MEAN	33.625		
FOR ENTIRE SAMPLE	42.357	19.749	168

*HEIGHT MEASURED FROM THE MEDIUM SURFACE TO THE APICAL MERISTEM.

TABLE 7

MEAN NUMBER OF NODES*PER PLANTLET GROWN FOR THIRTY DAYS UNDER LOW CALCIUM AND THREE LEVELS OF MAGNESIUM

STANDARD MG(1.5 mM)	MEAN	STD DEV	N
ATLANTIC	3.571	1.718	7
WF31-4	4.857	1.952	7
BELRUS	9.143	.900	7
KENNEBEC	5.429	1.718	7
MONONA	4.000	1.732	7
NORLAND	7.714	.488	7
RUSSETB	6.143	2.035	7
SUPERIOR	5.286	2.215	7
GRAND MEAN	5.768		
LOW MG(0.15 mM)			
ATLANTIC	2.714	1.380	7
WF31-4	3.143	1.069	7
BELRUS	6.000	1.000	7
KENNEBEC	3.714	1.254	7
MONONA	2.857	.690	7
NORLAND	3.143	1.464	7
RUSSETB	2.286	.488	7
SUPERIOR	3.143	1.069	7
GRAND MEAN	3.375		
TRACE MG(0.015 mM)			
ATLANTIC	2.143	.690	7
WF31-4	2.429	.535	7
BELRUS	5.143	2.854	7
KENNEBEC	2.857	.900	7
MONONA	2.286	.756	7
NORLAND	4.429	.535	7
RUSSETB	1.714	.488	7
SUPERIOR	2.714	1.113	7
GRAND MEAN	2.964		
FOR ENTIRE SAMPLE	4.036	2.226	168

TABLE 8

MEAN NUMBER OF LATERAL SHOOTS PER PLANTLET GROWN FOR THIRTY DAYS UNDER LOW CALCIUM AND THREE LEVELS OF MAGNESIUM

STANDARD MG(1.5 mM)	MEAN	STD DEV	N
ATLANTIC	3.000	1.826	7
WF31-4	2.143	1.345	7
BELRUS	0	0	7
KENNEBEC	3.714	3.638	7
MONONA	2.286	1.254	7
NORLAND	1.143	1.215	7
RUSSETB	4.857	2.911	7
SUPERIOR	4.429	1.512	7
GRAND MEAN	2.696		
LOW MG(0.15 mM)			
ATLANTIC	6.714	1.890	7
WF31-4	5.143	1.864	7
BELRUS	0	0	7
KENNEBEC	4.143	2.268	7
MONONA	5.714	1.799	7
NORLAND	4.143	2.478	7
RUSSETB	11.000	1.528	7
SUPERIOR	2.571	2.440	7
GRAND MEAN	4.928		
TRACE MG(0.015 mM)			
ATLANTIC	5.000	1.291	7
WF31-4	2.286	.756	7
BELRUS	.286	.488	7
KENNEBEC	1.143	.690	7
MONONA	4.429	3.690	7
NORLAND	1.286	1.113	7
RUSSETB	3.429	2.225	7
SUPERIOR	2.286	1.113	7
GRAND MEAN	2.518		
FOR ENTIRE SAMPLE	3.381	2.985	168

CONCLUSIONS

Calcium is known to be a required macroelement in the culture of all higher plants, whether the growth occurrs under sterile laboratory conditions or in the field. The requirements of individual species vary tremendously, each having its own range of tolerances to deficiency of this element. The preceding study has shown that there are significant differences within a single species, and that this variation can be recognized and quantified using tissue culture techniques. Of the eight clone lines tested, 'Superior' appears to be the most sensitive to calcium concentration in the medium. Even when grown under high calcium conditions it continues to show symptoms, suggesting a higher concentration of calcium is required for optimal growth of this variety in tissue culture. 'Russet Burbank', 'Kennebec', and 'Belrus' also show a high degree of sensitivity to low calcium levels, though they show greater improvement under the high level treatment. Sha *et al*. (1985) reported 'Norland' decreasing and then increasing in its

percentage of necrosis across the low, standard, and high calcium treatments. This correlates well with the results of this study. 'Atlantic' and 'WF31-4' react similarly to the calcium level treatments. Each variety responded differently to calcium levels in the media according to its own individual requirements.

Under conditions of low calcium, the effect of high magnesium concentration relative to calcium concentration exacerbating calcium deficiency symptoms was not observed. This is in direct opposition to data cited by Jones and Lunt (1985), stating that deformation attributed to calcium deficiency occurrs more readily at high magnesium levels. Progressive reduction of magnesium concentration in the growth medium of plantlets simultaneously stressed by low calcium levels yielded increased occurrences of abnormal growth in all varieties tested. This does not support the hypothesis that magnesium is in some way increasing the deleterious effects of low calcium. No variety showed a decrease in abnormality under reduced magnesium levels when subjected to reduced calcium levels. This is most likely due to a combination of stresses placed upon metabolism by deficiencies of both elements. The problems generated by the scarcity of both ions in the medium probably overshadows the

reduction of calcium-dependent symptoms elicited by the reduced magnesium. In order for the effects of a single element to be separated out of the gross malformations elicited by low levels of one or both elements, more finely tuned studies must be made of each participating element. These studies could possibly use electromicroscopic assays of tissue during early stages of malformation under carefully controlled deficiency situations. If in fact a synergistic relationship exists between calcium and magnesium levels, whether it be that high magnesium levels intensify calcium deficiency-related symptoms or magnesium is becoming toxic in some way at low calcium levels, then it cannot be supported by the data gathered by this study.

Since calcium is known to be closely associated with the transport of auxin (DeLa Fuente and Leopold 1973), and the formation and maintenance of properly functioning cell membranes (Marinos 1962), it is reasonable to suppose that this ion's scarcity would be felt most severely in a rapidly growing shoot apex and be manifested as tip necrosis.

Possible field applications for these data are indirect, owing to the vast differences in conditions between sterile tube culture and field growth.

However, the data gained from this study should serve as a starting point for field testing and evaluation of varieties in respect to calcium requirements. Varieties most likely to tolerate lower calcium levels in the soil and produce tubers would be those that showed the least abnormality under low calcium in tissue culture. Selection of these varieties for field testing would be a logical next step in the practical application of this information.

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