CHAPTER 5

EFFECT OF SOLUTION pH AND ITS INTERACTION WITH CALCIUM ON GERMINATION, EARLY VEGETATIVE AND REPRODUCTIVE GROWTH OF GROUNDNUT

5.1 INTRODUCTION

A low soil Ca level is one of the conditions often associated with acid-soils, particularly in the tropics (Sanchez, 1976; Von Uexkull & Mutert, 1995). This is in juxtaposition to the importance of Ca as a detoxifying agent of H^+, (Haynes, 1984; Foy, 1992) Al (Alva *et al.,* 1991; Mclay & Ritchie, 1993; Carvalho & van Raij, 1997) and Mn (Robson & Loneragan, 1970) toxicity to plants, also associated with many of the acid soils (Sanchez & Uehara, 1980; Clark, 1984; Foy, 1988, 1992; Noble *et al.,* 1988; Fageria *et al.,* 1990; Vaughan & Ord, 1991; Baziramakenga *et al.,* 1995). Arnon & Johnson (1942) concluded that the poor growth of lettuce, tomato and Bermuda grass grown in low solution pH was the result of a low Ca supply. Robson & Loneragan (1970) showed in a flowing solution culture study that Ca alleviates Mn toxicity on *Medicago* spp, while several studies have shown that high Ca concentrations in solution may alleviate Al toxicity in several legumes (Alva *et al.,* 1986; Cameron *et al.,* 1986; Munns, 1986; Noble *et al.,* 1986; Shamsuddin *et al.,* 1992).

A high concentration of H^+ ions in the soil solution is most consequential for legumes growing without N fertilizer as it affects rhizobial survival and multiplication in soils, and root infection and nodulation of the host plant (Andrew, 1978). Excess H^+ ions, because of the effects on nutrient uptake and retention by plant roots, can increase plant requirements for Ca and perhaps other nutrients in the growth medium (Foy, 1992). For groundnut, Ca deficiency results in blackened plumules, high incidences of pod rot and unfilled pods (pops), poor yields, inferior quality, poor seed germination, and disease susceptibility (Gascho & Davis, 1994). In acid soils, the direct effects of H^+ toxicity or absolute Ca deficiency on plant growth are difficult to determine because at soil pH<4.0, Al, Mn and other mineral elements may be present in toxic concentrations, and the availability of other elements essential for plant growth may be suboptimal (Foy, 1992). Because of

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these confounding effects, investigators use nutrient solutions or sand cultures to study the effects of low pH or Ca.

Investigations on the effect of Ca in alleviating acid-soil infertility for legumes have focused mainly on growth, nodulation, nitrogen fixation, nutrient uptake and mineral composition (Alva *et al,* 1990; Alva *et al,* 1991, van Rossum, *et al,* 1994; Tang & Thomson, 1996). Very little attention has been given to the effects of H^+ toxicity *per se* and Ca deficiency *per se* in the soil solution on germination and early seedling growth of legumes, particularly for groundnut. Because of their small size, seedlings are expected to be more vulnerable to acid-soil conditions. Studies that have investigated the effect of soil acidity at germination on yields of sweet clover and alfalfa (Haller, 1983), have shown that both crops grew well and produced large yields even on strongly acid soils (pH<4.0) provided that germination occurred in a neutral medium.

Groundnut is usually grown on light-textured soils that have a tendency to become acidic, but no attention has so far been given to effects of low soil pH in the pod-zone on groundnut pod development. Hence, it is not known to what extent low soil pH in the pod-zone affects groundnut productivity or to what extent the Ca applied in the pod-zone may alleviate soil acidity for the pods. This may be a topic that requires detailed research, and solution nutrient cultures can be more appropriate for further elucidation on the effects of solution pH and Ca on pod development.

5.2 MATERIALS AND METHODS

5.2.1 GERMINATION, SEEDLING SURVIVAL AND EARLY GROWTH

The objectives of the study were to examine the effect of pH and external Ca concentration on germination, seedling survival and early growth of groundnut in sand culture. Four growth chamber experiments were conducted between June and September 2000 at the Experimental farm of the University of Pretoria. Germination and early growth of the short season S*panish* groundnut cv. *Falcon* were tested for response to solution pH that was varied independently or in factorial combination with solution Ca concentration.

Experiment 1: Effect of pH on germination

In this experiment, the effects of four pH levels (3.0, 4.0, 5.0 and 6.0) on germination of groundnut were investigated. Healthy groundnut kernels that had been produced under conditions of adequate Ca nutrition were germinated between paper towels on acid-washed sand in seedling trays, and kept at 27^{o}C and 100% relative humidity under a 16-hr photoperiod. The sterilised sand was moistened with a dilute nutrient solution comprising (Φ*M*) 250 K, 250 N, 300 Ca, 400S, 100 Mg, 10 Fe (as EDTA), 10 Cl, 3B, 0.25 Zn, 0.10 Mn, 0.07Cu and 0.02 Mo. This solution had a pH of 6.5, and was titrated with either 0.1*M* H_2SO_4 or 0.1*M* HCl to obtain the target treatment pH values.

There were four replicates consisting of 100 seeds per treatment combination, resulting in a total of 400 seeds per pH treatment. The treatments were arranged in a randomized complete block design. Seeds showing radicle emergence (5 mm) were recorded as germinated (Mayer & Poljakoff-Mayber, 1975), and the germinated seeds were counted every day during the five-day experimental period. The experiment was repeated four times, twice acidifying the nutrient solution with H_2SO_4 and twice with HCl.

Experiment 2: Effect of pH on seedling growth

The pH treatments were similar to those in Experiment 1. For each treatment combination, twenty five pre-germinated kernels were planted 2.5 cm deep in acid-washed sand contained in 35 x 30 x 15 cm deep seedling trays. The sterilised sand was kept moist by periodic irrigation with the same nutrient solution as described in Experiment 1. The experiment was conducted with four replications.

After emergence, the seedlings were allowed to grow for 21 days during which seedling mortality was assessed at 7-day intervals. At 21 days after emergence, the surviving healthy plants were harvested, and roots were separated from their tops. Root length and root surface area were estimated using a GLS root scanner (HP Scanjet 3C). The roots were classified into three diameter categories: (a) roots with diameter <1.0 mm, (b) roots with diameter 1.0 B 2.0 mm, and (c) roots with diameter >2.0 mm. The root surface area was measured in mm^2. The plant tops and roots were oven-dried at 80^{o} C for 48 hrs to determine dry mass. The experiment was repeated

four times, twice acidifying the nutrient solution with H_2SO_4 and twice with HCl.

Experiment 3: Effect of pH and Ca on germination

Effects of three solution pH levels (pH 3.5, 4.5 and 5.5) and five levels of solution Ca concentration (0, 50, 100, 150 and 200 Φ*M* Ca) in factorial combination on germination were investigated. The basal nutrient solution was as in Experiment 1, but without the Ca. The desired Ca concentrations were obtained by adding the appropriate amounts of $CaSO_4$.$2H_2O$ to the nutrient solution. Since Ca was added to the nutrient solutions as the $CaSO_4$ salt, the concentration of S in the nutrient solution also varied from 100 Φ*M* in solutions that contained no Ca to 300 Φ*M* in those that contained 200 Φ*M* Ca. There were four replicates consisting of 100 seeds per treatment combination, resulting in a total of 400 seeds per treatment. The germination conditions were similar to those in Experiment 1, and the experiment was repeated four times.

Experiment 4: Effect of pH and Ca on seedling growth

The factorial pH and Ca combination treatments and replicates in this experiment were identical to those used in Experiment 3. Twenty five pre-germinated seeds per treatment combination were used. The experimental techniques were as in Experiment 2. At 21 days after emergence root length, root surface area and dry mass of the roots and shoots were determined. The experiment was also repeated four times.

Data Analysis

Since there were no differences in effects between solutions titrated with H_2SO_4, and those titrated with HCl, the results were combined for analysis. Analysis of variance (ANOVA) was performed using the General Linear Model procedure provided by the Statistical Analysis System (SAS, 1996). If ANOVA determined that the effects of the treatments were significant ($P \leq 0.05$), the treatment means were separated by Duncan's Multiple Range test.

5.2.2 REPRODUCTIVE GROWTH

Two growth chamber experiments were conducted between December 2001 and May 2002 at the Hatfield Experimental Farm of the University of Pretoria. Pod initiation and development of the

short season S*panish* groundnut cultivars *Falcon* and *Jesa* were tested for response to solution pH that was varied independently or in factorial combination with solution Ca concentration.

Experiment 5: Effect of pH on pod development

The effects of five solution pH values (pH 3.0, 4.0, 5.0, 6.0 and 7.0) on pod initiation and development were investigated. Seeds of cultivar *Jesa* were planted 2.5 cm deep in a moist coir/sand mix contained in rectangular PVC crates of 58 x 48 x17 cm. The seeds were planted in two rows spaced 35cm apart, with an in-row spacing of 10cm. Ten days after emergence the plants were thinned to six per crate (three per row). Throughout the experiment the plants were drip-irrigated with a complete nutrient solution, and vigorous plant growth with no apparent water or nutrient stress was observed. The nutrient solution contained (μM) 300 N, 2 P, 250 K, 300 Ca, 400 S, 100 Mg, 10 Fe (as EDTA), 10 Cl, 3B, 0.25 Zn, 0.10 Mn, 0.07Cu and 0.02 Mo. Following the methodology of Zharare *et al.,* (1998) flowers and gynophores produced close to the base of the plants were removed to encourage flowering higher up the plant for experimental convenience.

At gynophore initiation ten glass test tubes were buried in the sand around each plant. Gynophores of approximately the same age that were $\forall$5 cm long were individually positioned in 15ml of a simplified nutrient solution. The glass tubes were loosely covered with aluminium foil to allow adequate aeration but exclude light. The simplified nutrient solution containing only Ca, S, Fe and Zn was used to produce normal and healthy pods by Zharare (1997). The composition of the nutrient solution was (ΦM) 500 Ca, 540 S, 4 Fe (Fe-EDTA) and 0.5 Zn. This solution was titrated with 0.1M H_2SO_4 or KOH to obtain the target treatment pH values. The nutrient solution was refreshed daily for the five weeks during which the pods were cultured. A vacuum pump was used to suck out the solutions from the glass tubes. Refilling each tube with 15 ml of nutrient solution was done with the aid of a calibrated dispenser. An automatic irrigation system controlled by a Richdel irrigation controller was used to supply nutrient solution to the root zone.

The test tubes were inspected daily to establish the time to initial pod expansion (basal and apical seed compartments). The number of cultured gynophores that produced normal pods was recorded after five weeks (35 days), so were pod fresh and dry mass and kernel dry mass. Kernels from these

pods, as well as from pods that were allowed to develop in the sand medium were analysed for Ca content. For experimental convenience, each crate represented one pH level, and each of the six plants per crate was considered to be a replicate. The placement of ten tubes per plant resulted in a total of 60 gynophores per pH treatment.

Experiment 6: Effect of pH and Ca on pod development

Cultivar *Falcon* was used in the experiment, and the planting arrangements and crop management were similar to Experiment 5. The effects of three solution pH levels (pH 3.5, 5.0 and 6.5) and three levels of solution Ca concentration (500, 1000 and 2000 Φ*M* Ca), in factorial combination, on pod development were investigated. The treatments were arranged in a split plot design, with pH level as the main plots and Ca level as the sub-plots. There were three plants per treatment combination, and each plant was considered to be a replicate. Thirty gynophores per treatment combination were cultured.

The technique of culturing gynophores resembled that described in Experiment 5, and the nutrient solution was also similar to that used in Experiment 5, but without the Ca. The desired Ca concentrations were obtained by adding the appropriate amounts of $CaSO_4$.$2H_2O$ to the nutrient solution. Records taken in the experiment were similar to those described in Experiment 5.

Scanning electron microscopy

In both experiments, submerged portions of gynophores/pods were sampled, and the surface tissue checked for development of hairs. Mature seeds were sectioned longitudinally; one cotyledon of the seed was removed, leaving the plumule, hypocotyl and radicle intact and attached to the other cotyledon. Examinations of the excised seeds were carried out to determine embryo development at different Ca concentrations and at different pH levels using scanning electron microscopy.

Representative samples of gynophores/pods from each treatment were harvested at intervals during the experiment for microscopic analysis. The samples were immediately fixed in 2.5% gluteraldehyde in 0.075*M* phosphate buffer (pH 7.4) for 48 hours. The specimens were then

rinsed three times in the same buffer for 15 minutes per rinse. Dehydration of the samples in ethanol was in an ascending series: 50%, 70% and 90% for 10 minutes each. The samples were finally dehydrated three times for 15 minutes per dehydration in fresh 100% ethanol before being dried in a Biorad critical point drier (Biorad, Polaron Division, Watford, England). After drying, the specimens were mounted on aluminium stubs and coated with gold in a Polaron E5200 autocoating unit (Polaron Equipment Ltd, Watford, England). Specimens of the gynophores/pods were examined using a JSM-840 scanning electron microscope (JEOL, Tokyo, Japan) equipped with a Tracor image analysis system. Specimens of the seeds were examined with a light microscope (Nikon SMZ 800 stereo microscope) equipped with a Nikon DXM 1200 digital camera. The work was conducted in the Laboratory for Microscopy and Micro-analysis, University of Pretoria.

Data Analysis

Analysis of variance (ANOVA) was performed using the General Linear Model procedure provided by the Statistical Analysis System (SAS, 1996). If ANOVA determined that the effects of the treatments were significant ($P \leq 0.05$), the treatment means were separated by Duncan's Multiple Range test.

5.2 RESULTS AND DISCUSSION

5.2.1 GERMINATION, SEEDLING SURVIVAL AND EARLY GROWTH

Experiment1: Effect of pH on seed germination

The germination of groundnut seed was less sensitive to the effects of solution pH on day two compared to days three and five (Table 5.1). The proportion of germinated seeds increased with time in all solution pH treatments during the 5-day experimental period, though at a lower rate in the pH 3.0 treatment. By the end of the germination period, the number of germinated seeds was similar at pH 4.0, 5.0 and 6.0, but lower at pH 3.0. The final germination count on day five ranged from 86% at pH 3.0 to 93% at pH 6.0.

From an agronomic point of view, the faster the seedling emerges the greater the likelihood of escaping pre-emergence diseases, and the less damage will be exacted by seed and seedling pathogens (Melouk & Backman, 1995). Thus, the slower germination observed at pH 3.0 may

make the imbibed seeds in the soil more vulnerable to fungal and bacterial pathogens in the soil, leading to reduced seedling emergence.

Table 5.1 Effect of pH on germination of groundnut during a 5-day experimental period, and on seedling survival during a 21-day experimental period

Treatment	Experiment 1 Germination (%)			Experiment 2 Seedling survival (%)		
	Day 2	Day 3	Day 5	Day 7	Day 14	Day 21
PH 3.0	30	74	86	72	61	51
PH 4.0	31	81	91	84	83	78
PH 5.0	32	85	92	87	87	85
PH 6.0	32	84	93	97	94	92
Mean	**31**	**81**	**91**	**85**	**81**	**77**
LSD $_{(0.05)}$	**6.0**	**6.3**	**3.0**	**16**	**18**	**18**

**Each value is the average of 16 replicates.

Experiment 2: Effect of pH on seedling growth

All the pre-germinated seeds emerged from the sand, but the number of surviving seedlings at all pH levels gradually declined, starting 7 days after emergence (Table 5.1). Only 51% of the seedlings survived to 21 days after emergence in the pH 3.0 treatment, compared to 92% in the pH 6.0 treatment. In general, seedling survival improved as the pH increased.

The total root length at 21 days after emergence was 1.65 m per plant for plants grown at pH 3.0, and increased by 526% to 8.67 m for plants grown at pH 6.0 (Figure 5.1). At pH 4.0, the total root length was similar to that obtained at pH 5.0. Roots thicker than 2mm in diameter in the pH 3.0 and pH 4.0 treatments exhibited visual symptoms similar to those reported by Lund (1970) for H^+ injury on plant roots. These symptoms included stunted root growth, brownish colour and little lateral root development. Some of the roots were decayed. Root browning has been attributed to enhanced suberization, which may limit water (and nutrient) uptake (Barceló & Poschenrieder, 1990).

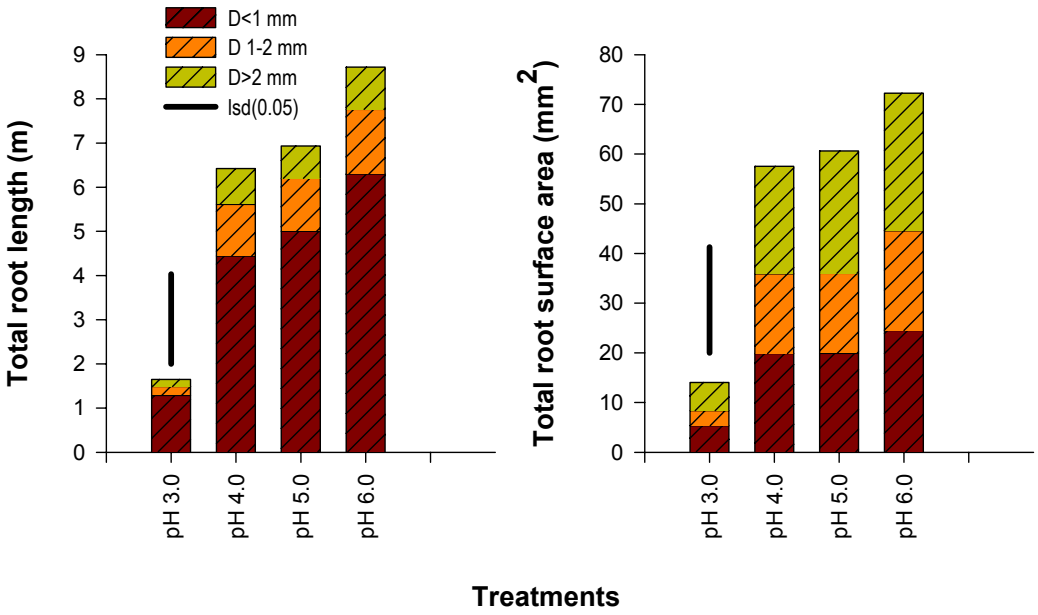


Figure 5.1 Effect of solution pH on total root length and total root surface area of groundnut at 21 days after mergence

Hydrogen-induced root injury may change root membrane permeability (membranes become leaky), interfere in absorption and transport of nutrients, increase loss of organic substrates (sugars, amino acids) and adsorbed cations, and reduce capacities for absorption of nutrients (Foy, 1992). Thus, plants grown in acid soils are bound to be restricted from utilising available water and nutrients when root proliferation and root function is limited by low pH (Goldman, 1989; Sanzonowicz *et al.,* 1998).

Root surface area for individual root diameter categories followed the same response trends as root length (Figure 5.1). At each root diameter category, root surface area increased by more than 300% for plants growing at pH 6.0 compared to those growing at pH 3.0. Total root surface area also increased with increasing solution pH. A large increase in total root surface area was observed in the pH 6.0 treatment (72 cm^2) compared to the pH 3.0 treatment (14 cm^2).

The detrimental effects of a solution pH of 3.0 on the shoots were evident within ten days of plant growth, when the shoot growth was visibly impaired and the leaves had a greyish-green colour. At three weeks after emergence, shoot dry mass increased in response to solution pH increases

(Table 5.2). The shoot dry mass was not significantly different at pH 5.0 and 4.0, although plants in the latter treatment displayed some symptoms of H^+ injury.

Table 5.2 Effect of pH on shoot and root dry mass (g plant^{-1}) of groundnut seedlings at 21 days after emergence

Treatment	Shoot dry mass (g plant^{-1})	Root dry mass (g plant^{-1})
pH 3.0	0.270	0.046
pH 4.0	0.372	0.052
pH 5.0	0.392	0.076
pH 6.0	0.460	0.096
Mean	**0.374**	**0.068**
LSD (0.05)	0.047	0.009

**Each value is the average of 16 replicates.

The effect of solution pH on root dry mass was analogous to that on shoot dry mass, only slightly more adverse (Table 5.2). Root dry mass increased in response to solution pH increases, ranging from 0.046 g plant^{-1} at pH 3.0 to 0.096 g plant^{-1} at pH 6.0. The adverse effects of pH on root dry mass were comparable at the lower pH levels (pH 3.0 and 4.0.). More severe depressions in root growth compared to shoot growth of some grain legume species grown at low pH have been observed (Jayasundara *et al.,* 1998), and attributed to decreased proton extrusion from the roots (van Beusichem, 1982; Schubert *et al.,* 1990; Yan *et al.,* 1992), which may lead to limited nutrient uptake. Van Beusichem (1982) observed a 40% reduction in root dry mass of field pea grown at pH 5.5 without a reduction in shoot dry mass. In the present study, a 53% reduction in root dry mass was observed when the pH decreased from pH 6.0 to pH 3.0, compared to 41% reduction in shoot dry mass at the same pH levels.

The adverse effects of low solution pH were greater on root surface area compared to root dry mass. More fine roots developed at high pH levels compared to the low pH levels (pH 3.0 and 4.0), where short and stubby roots were prominent. This phenomenon can be attributed to inadequate Ca uptake, which negatively impacts on cell division and elongation, resulting in a shorter and denser root system (Clarkson, 1984; Wild *et al.,* 1989; Yan *et al.,* 1992). It is

probable that low pH may trigger the redirection of more assimilates to the roots than to the shoot system in order to offset the adverse effects of unfavorable pH on root growth. This would result in the accumulation of assimilates in the roots, giving rise to the formation of short and stubby roots, hence the smaller differences in root dry mass at the different pH levels compared to the root surface area.

Experiment 3:Effect of pH and Ca on seed germination

At day two, only 50% of the kernels germinated at Ca concentrations <0.5m*M* (Figure 5.5). As the Ca concentration increased, the negative effect of pH diminished and germination improved. At day five, germination percentages of more than 92% were obtained with Ca concentrations of 1.0m*M* or higher, irrespective of the pH (Figure 5.2). At Ca concentrations of less than 1.0m*M,* germination percentages were lower, especially at pH 3.5.

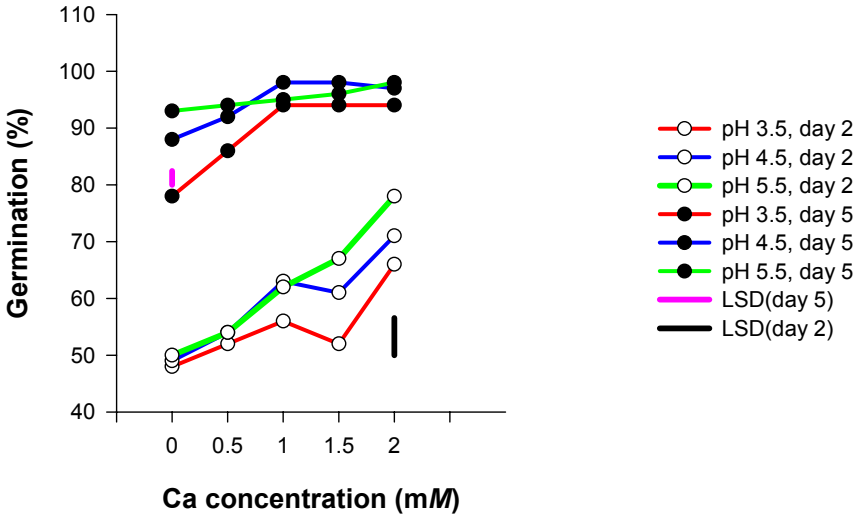


Figure 5.2 Effect of solution pH and Ca concentration on germination of groundnut

Experiment 4: Effect of pH and Ca on seedling growth

At 21 days after emergence, the solution pH and Ca concentration had significant interaction effects on seedling survival (Figure 5.3). At Ca concentrations less than 1.0 mM, there were large effects of pH on seedling survival, with survival percentages decreasing as the pH levels decreased. At pH 5.5 more than 95% of the seedlings survived, regardless of the Ca concentrations of the nutrient solution. At pH 3.5 seedling survival was similar to that obtained at

a pH of 5.5, provided adequate Ca was supplied. As the concentration of Ca decreased, seedling survival decreased to 80% with 0.5 m*M* Ca, and to 68% with no Ca in the solution. Seedling survival at pH 4.5 was intermediate to the responses observed at pH 5.5 and pH 3.5.

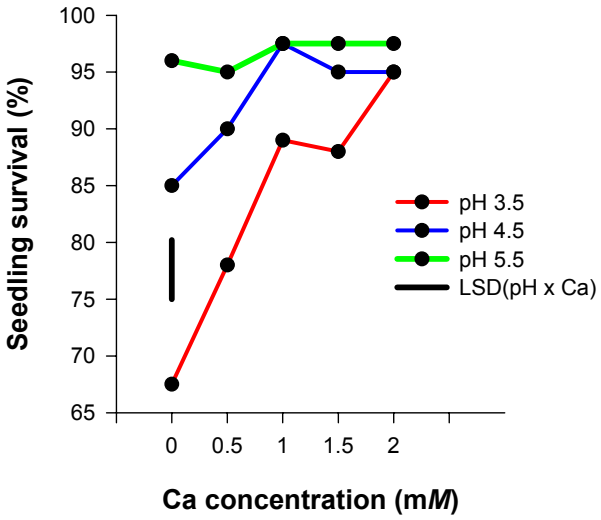


Figure 5.3 Effect of solution pH and Ca concentration on seedling survival of groundnut

The interaction effects of pH and Ca concentrations in the solutions were significant for root length for all root diameter categories (Figure 5.4). In the absence of Ca the length of roots increased when pH was increased from 3.5 to 5.5, and the increases were highest with roots of diameter <1.0 mm. Increasing the solution Ca concentration from 0.5 m*M* to 2.0 m*M* at pH 3.5 resulted in increases in root length of up to 104% for roots of diameter <1.0 mm. By comparison, the increases in root length (diameter < 1.0mm) over the same range of Ca increments were up to 133% at pH 4.5 and 92% at pH 5.5. At all pH values, root length generally increased with increases in solution Ca concentrations. The total root length per plant was highest (7.82 m) for plants grown at pH 5.5 with 2.0 m*M* Ca. Root formation in solutions without Ca was generally impeded.

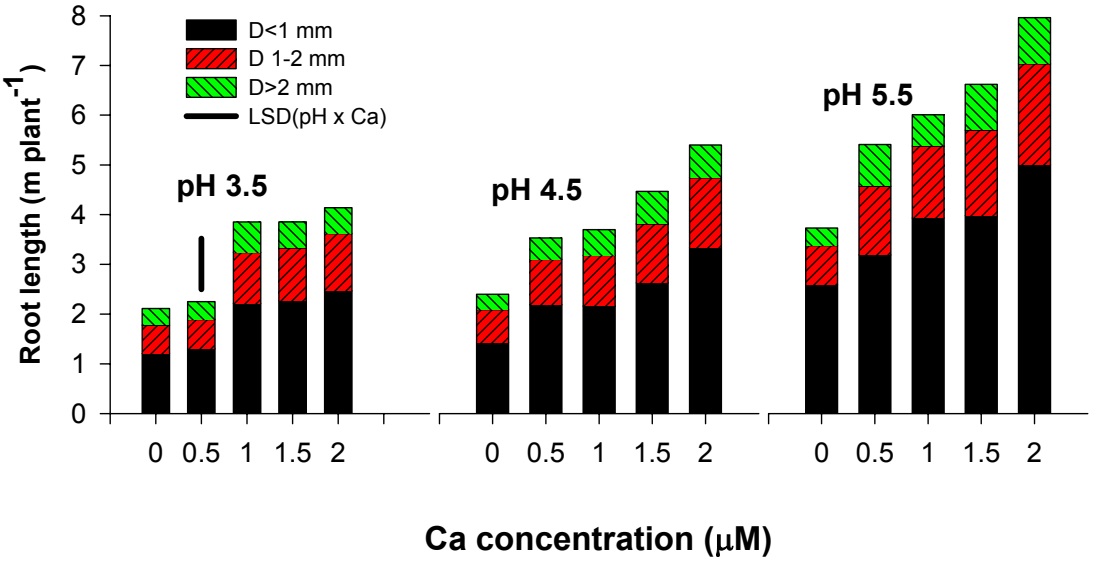


Figure 5.4 Effect of solution pH and Ca concentration on root length of groundnut at 21 days after emergence

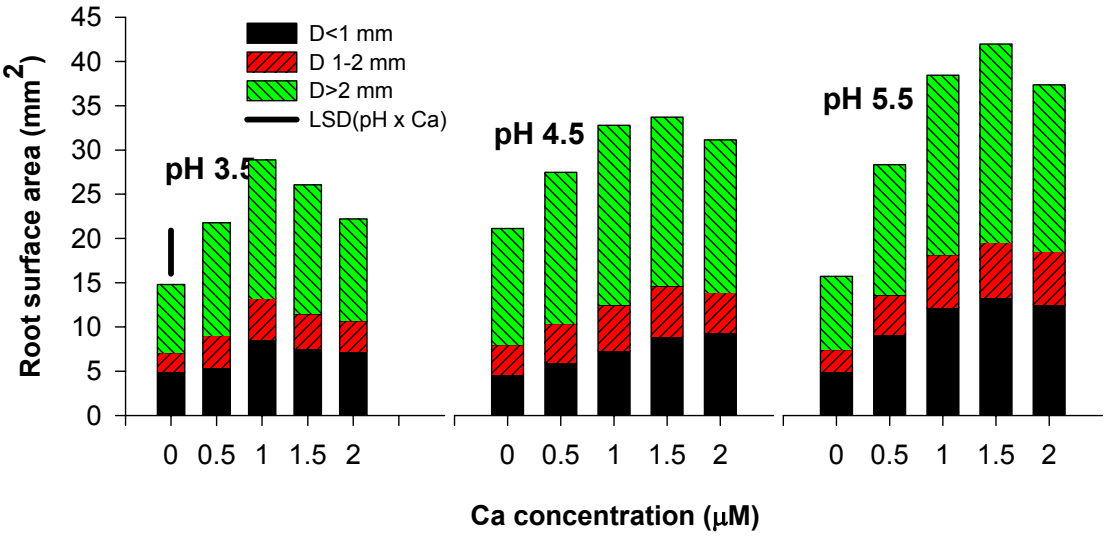


Figure 5.5 Effect of solution pH and Ca concentration on root surface area of groundnut at 21 days after emergence

Root surface area followed the same response trends as root length (Figure 5.5). In the absence of Ca, solution pH did not have an effect on root area for roots with diameter <2mm, whereas root surface area for roots with diameter > 2mm increased with increases in pH. With application

of Ca, root surface area for all root categories increased with increases in solution Ca concentrations. The increases were highest with 1.0 m*M* Ca at pH 3.5, and with 1.5 m*M* Ca at pH 4.5 and pH 5.5. The total root surface area at pH 5.5 was 95% higher than at pH 3.5. When averaged across solution pH treatments, increasing the Ca concentration from 0 to 2.0 m*M* Ca increased total root surface area by 76%. However, significant pH x Ca interactions occurred as can be seen in Figure 5.5. With application of Ca, total root surface area increased with increases in solution Ca concentrations up to a concentration of 1.0 m*M* Ca at pH 3.5 and up to 1.5 m*M* Ca at pH 4.5 and 5.5.

In this experiment, it was observed that there was a greater reduction in the development of the finer roots than of the thicker roots and taproot at the lower pH levels and lower Ca concentrations. Sanzonowicz *et al.* (1998) documented that H^+ toxicity inhibited the length of lateral roots of soybeans more than that of taproots. In their study, a 50% reduction in lateral root length occurred at pH 5.1, whereas a similar reduction in taproot length occurred at pH 4.7.

The effects of pH and Ca concentrations on shoot dry mass were significant, with shoot dry mass increasing as pH and Ca increased (Figure 5.6). The interaction effects between pH and Ca concentrations were also significant, showing a greater impact of pH on shoot dry mass at intermediate Ca concentrations. Plants grown with solution Ca concentration of 2.0mM produced similar dry mass at the three pH levels. The shoot dry mass at pH 3.5 was reduced from 0.48 g plant^{-1} at 2.0m*M* Ca to 0.29 g plant^{-1} with no Ca in the solution. Yan *et al.,* (1992) documented similar results in their studies on maize and broad beans, which showed that higher levels of solution Ca counteracted the negative effects of low solution pH on growth of the two crops.

Better root growth was observed when the solution pH was favourable (Figure 5.6). The root dry mass was highest at pH 5.5, and ranged from 0.04 g plant^{-1} with no Ca to 0.09 g plant^{-1} at 2.0m*M* Ca. At a pH of 3.5, root dry mass ranged from 0.04 g plant^{-1} at 0 Ca to 0.05 g plant^{-1} at 2.0m*M* Ca. Root dry mass responses at a pH of 4.5 were intermediate to those observed at pH 5.5 and pH 3.5. The interaction effects of solution pH and Ca concentration on root dry mass were significant, showing smaller increases in root dry mass at Ca concentrations <1.0 1.0 m*M*, but significant increases at Ca concentrations > 1.0 m*M*.

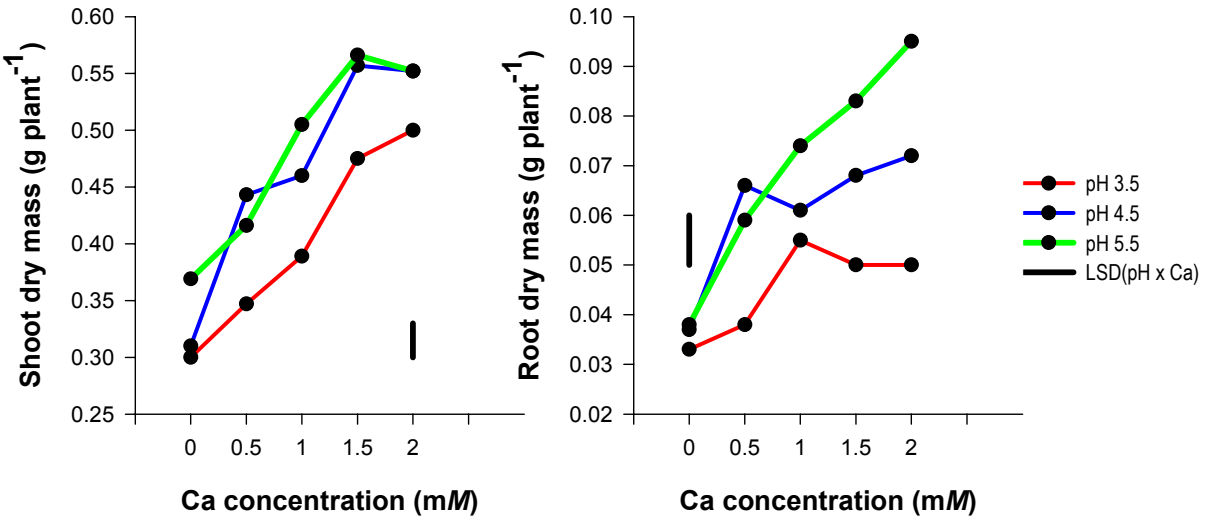


Figure 5.6 Effect of solution pH and Ca concentration on shoot and root dry mass of groundnut at 21 days after emergence

The results showed that the adverse effects of pH on root growth were more at low solution Ca concentration. This would be expected because competition with H^+ on absorption at low pH may induce Ca deficiency, resulting in inhibited root growth since Ca is needed for mitosis and cell elongation (Rost-Siebert, 1985). Koyama *et al.* (2001) observed that the roots of *Arabidopsis thaliana* growing at pH 5.0 required less Ca to maintain elongation compared to those growing at pH 4.5 or 4.8, and concluded that the amelioration of low-pH damage by application of Ca demonstrated the involvement of a Ca-requiring process.

5.3.2 REPRODUCTIVE GROWTH

Experiment 5: Effect of pH on pod development

For both the apical and basal seed compartments, the time to initial pod expansion was significantly affected by solution pH. At solution pH 5.0, 6.0 and 7.0, the expansion of the basal compartment started at approximately six days after submergence of the gynophores (Table 5.3). In the pH 4.0 treatment visible pod development was observed 11 days after submergence of the gynophores. There was a marked delay in pod development in the pH 3.0 treatment, with initial expansion of the basal compartment being visible 16 days after submergence of the gynophores.

Table 5.3 Pod formations and time to visible pod expansion of groundnut *cv* Jesa cultured in nutrient solution at different pH levels

Treatment	Days to initial pod expansion (basal seed compartment)	Days to expansion of apical seed compartment	% Cultured gynophores that produced normal pods
pH 3.0	16.3±1.3	ND	11.7±7.65
pH 4.0	11.0±1.0	11.9±0.89	55.0±3.31
pH 5.0	6.9±0.85	8.1±0.52	91.6±2.4
pH 6.0	5.5±0.2	6.5±0.5	95.0±1.0
pH 7.0	6.1±0.5	7.9±0.37	93.3±0.9
Mean	**9.2**	**8.6**	**69.3**
LSD $_{(0.05)}$	1.71	1.60	10.63

* *Data are means of six replicates ± standard deviation ND - not detected

Expansion of the apical seed compartment was even more sensitive to solution pH (Table 5.3). Rapid expansion (6.5 days after expansion of the basal seed compartment) was observed at pH 6.0, followed by pH 7.0. It took 12 days for expansion of the apical seed compartment to commence at pH 4.0. In the pH 3.0 treatment no expansion of the apical pod compartment was observed at termination of the experiment, 35 days after submergence of the gynophores.

The percentage of gynophores that developed into pods ranged from 95% at pH 6.0 to 12% at pH 3.0 (Table 5.3). While pod expansion was initiated by all the 60 gynophores submerged in solution with pH 3.0, only seven (12%) showed visible pod expansion and developed into one-compartment pods. At pH 4.0 the number of gynophores that developed into pods increased to 55%. These were mainly two-compartment pods as in the rest of the pH treatments. Symptoms attributed to H^+ injury were observed on pods formed at pH 4.0. The pods showed patches of brown discoloration typical of H^+ injury. Similar symptoms were observed by Zharare (1997) on pods grown in nutrient solutions containing no Ca. Pod-set in gynophores cultured at solution pH 6.0 and 7.0 was >90%, with the highest number of pods being formed at pH 6.0. Better pod growth was observed when the solution pH was favourable (Figure 5.7).

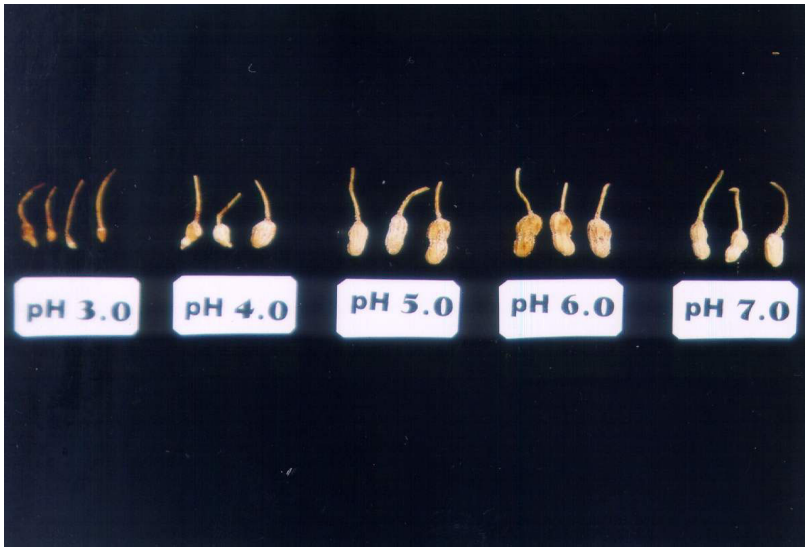


Figure 5.7 Effect of pH on pod development

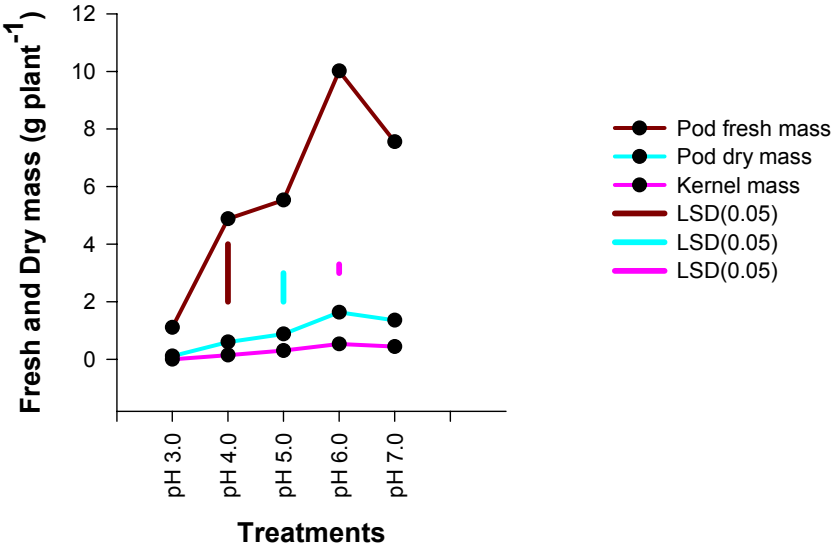


Figure 5.8 Pod fresh and dry mass, and kernel weight of groundnut *cv* Jesa cultured in nutrient solution at different pH levels

Pod fresh and dry mass increased in response to solution pH increases up to pH 6.0, but the increases were larger for the fresh than for the dry mass (Figure 5.8). Whereas the pod fresh mass at pH 7.0 was significantly lower than that at pH 6.0, the dry mass observed at both pH

levels was not statistically different. Although the kernels were not physiologically mature at the time of harvest, kernel dry mass showed significant effects of solution pH (Figure 5.8). Kernel dry mass increased with increasing solution pH up to pH 6.0, and ranged from 0.15 g kernel^{-1} at pH 4.0 to 0.54 g kernel^{-1} at pH 6.0. There was a 17% reduction in kernel dry mass at pH 7.0 relative to pH 6.0.

The poor pod development at low pH can be explained in terms of the inhibitory effects of low pH on proton release which is perceived as the driving process for the uptake of nutrient cations (Leonard, 1984; Briskin, 1986). Since the groundnut gynophores and developing pods absorb nutrients directly from the soil (Skelton & Shear, 1971; Beringer & Taha, 1976; Chahal *et al.,* 1979), proton release should also be the driving process for the uptake of nutrient cations. It is therefore possible that the high H^+ activity in the low solution pH resulted in a net H^+ influx into the pods, which in turn led to limited nutrient uptake and pod growth. There are striking similarities in the symptoms of low pH injury obtained in this study, and those of Ca deficiency obtained by Zharare (1997), suggesting that Ca deficiency may have played a role in the impairment of pod growth at low pH. Bledsoe *et al.,* (1949) documented that Ca requirements for pod growth are greatest at the start of gynophore swelling, and any deficiency in Ca at this stage results in failure of gynophores to expand into pods. Nonetheless, the restricted pod growth at low solution pH in this study could have been the direct effects of proton toxicity as a result of high proton influx into the pods

White patches showed on the surfaces of gynophores cultured at all pH levels, starting from approximately 48 hours after submergence until the pods were harvested (Figure 5.9). Normal developing gynophores have minute white hairs that give a downy appearance (Seshadri, 1962), and are shed as the pods mature. Zharare (1997) observed the appearance of these patches approximately 14 hours after the gynophores had been submerged in nutrient solution, and these were tufts of hair covered with mucilage. Developing gynophores may bear unicellular structures resembling root hairs that can reach very high density and lengths of up to 0.75 mm (Zharare *et al.*, 1993; Gascho & Davis, 1994). In this study an examination of these white patches showed some fungal hyphae, especially at pH 3.0 and to a lesser extent pH 4.0 (Figure 5.10). The presence of fungal infection at low pH is not surprising, since fungi in general seem to dominate acid soils more than bacteria because they have hyphae and thicker cell walls that may make them more adaptable at lower soil pH (Bezdicek *et al.,* 2002).

Figure 5.9 Developing pod showing white patches

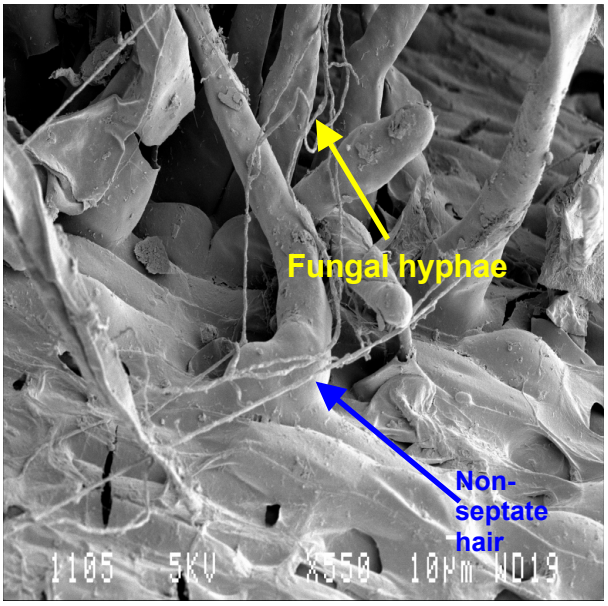


Figure 5.10 Scanning electron micrograph of gynophore surface showing non-septate hairs and fungal hyphae at pH 3.0

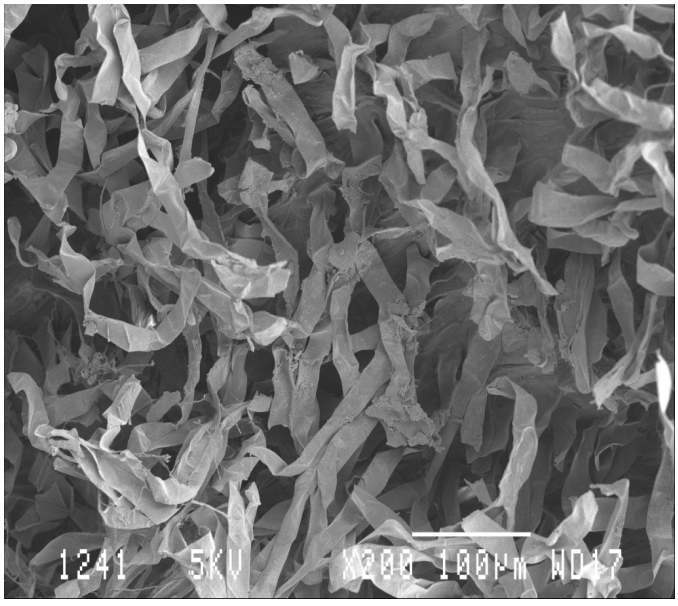


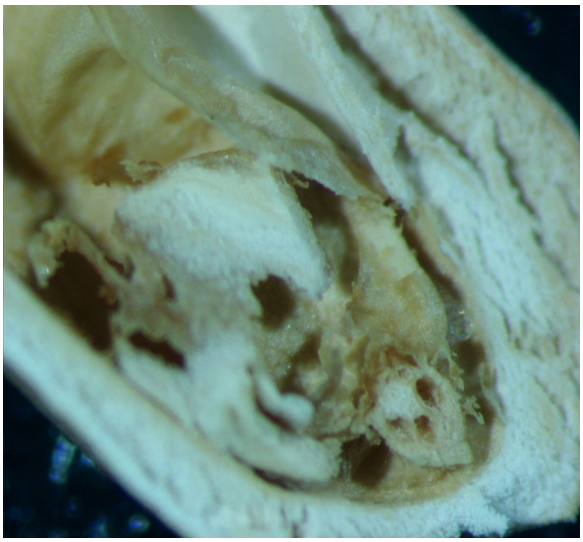
Figure 5.11 Scanning electron micrograph showing dense hair formation on gynophore surface cultured at pH 5.0.

Solution pH did not influence pod hair development since well-developed septate and non-septate hairs were observed even at pH 3.0. As described by Zharare (1997), the non-septate hairs arose as outgrowths of the primary epidermis, and had swollen bases (Figure 5.10). After shedding of the primary epidermis, branched, septate hairs were revealed. Literature on the effect of pH on groundnut gynophore and pod hairs is scarce. In other plant species, root hair development has been shown to be associated with acidification of the apoplast, for example in *Arabidopsis thaliana* (Bibikova *et al.,* 2001). The authors observed acidification at the root hair initiation site, and this acidification was maintained to the point where the process of root hair initiation ceased and tip growth began. They concluded that localized changes in apoplastic and cytoplasmic pH are associated with root hair development. It is probable that gynophore hair development is also associated with this phenomenon, hence the occurrence of peg hairs at pH levels as low as pH 3.0.

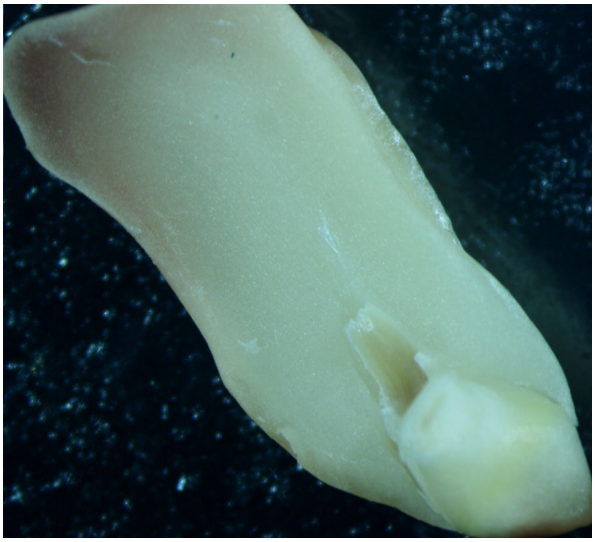
Although hair formation on gynophore surfaces was observed at all pH levels, the degree of hairiness appeared to decline with time at a faster rate with increased acidity. The results imply that while peg hair initiation can take place at low pH, its persistence (longevity) may be curtailed at low pH. Dense hair formation was observed at pH 5.0 and higher (Figure 5.11). Since the pod is capable of direct nutrient absorption from the soil, the density of hairs on the surfaces of developing pods might be expected to influence the uptake of Ca and other nutrients by the developing pods. If dense hair formation implies better nutrient uptake because of increased area of the absorption surface, the results would imply that better nutrient uptake could be expected at higher solution pH levels. Wissuwa & Ae (2001) observed that root hair density, as well as the ability of groundnut genotypes to form root hairs correlated with the presence and density of hairs on gynophores, suggesting a substantial increase in the surface area of roots and pods, which leads to increased nutrient uptake efficiency.

Kernel development

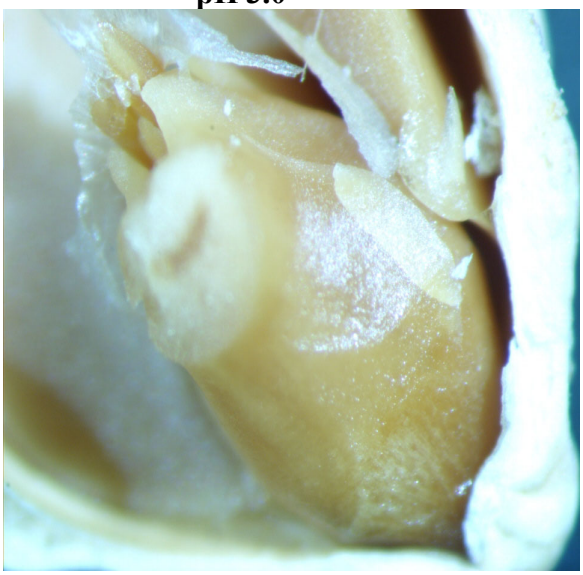
Microscopic examinations of the excised seeds showed that normal embryos were formed at pH 5.0 and above, and plumule development appeared to improve as the pH levels increased, with no differences in the overall appearance of the embryonic plumules being observed between pH 5.0 and 7.0 (Figure 5.12).



pH 3.0

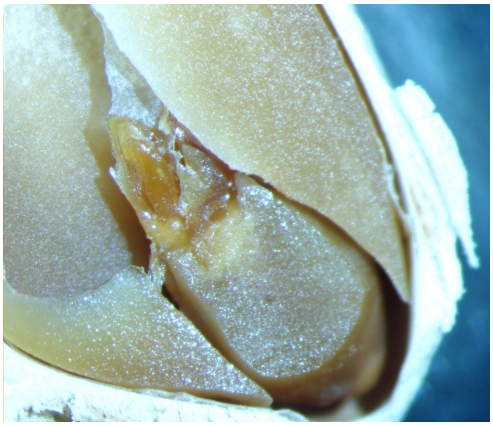


pH 4.0



pH 5.0

pH 6.0



pH 7.0

Figure 5.12 Photomicrographs of cotyledons and embryos produced at different pH

Seeds with slightly discolored cotyledons and a hollow area in the cotyledon were observed at pH 4.0. This abnormality is referred to as hollow heart, and is associated with Ca and B deficiencies (Harris & Brolmann, 1966). At pH 3.0 the whole embryonic axis (plumule, hypocotyl and radicle) was necrotic and largely undifferentiated. These symptoms were similar to those described by Zharare (1997) for groundnut TMV-2 pods grown in nutrient solution lacking Ca. Whatever the mechanism involved, it appears that the injuries caused by low pH (high H^+ activity) and low Ca to the developing groundnut embryo could be inter-linked. Zharare (1997) hypothesized that injuries to developing pods and kernels at low Ca in the pod environment could be caused by H^+ toxicity because of an enhanced H^+ influx into the pods from the pod environment in response to K^+ efflux. Zharare (1997) further hypothesized that one of the major functions of Ca in the pod environment is to prevent H^+ toxicity to developing pods by substituting for H^+ influx associated with the K^+ efflux. Furthermore, there could be mutual uptake inhibition between Ca and H^+ (Haynes, 1984; Foy, 1992). Thus, the symptoms of injury from high H^+ and Ca deficiency could be similar, as is the case with respect to root-tip growth (Lund, 1970). Hence, the necrosis of the embryonic axes at low pH could be the result of direct toxicity of H^+ activity or could be a result of Ca deficiency. Competitive effects of nutrients (soluble NH_4, K, Mg and Na salts) in the pod zone can also cause Ca deficiency or pod rot to develop (Csinos, 1986). Furthermore, the involvement of fungal pathogens in necrosis of the embryonic axis may not be precluded, since some fungal hyphae were observed on surfaces of gynophores growing at pH 3.0 and pH 4.0.

The effects of pH on seed quality were distinct, as evidenced by the embryo characteristics at the various pH levels (Figure 5.12). Though same age gynophores were cultured, the plumules after 40 days of pod growth were at different stages of development, with more advanced development being observed at solution pH 5.0 and higher. Thus, spatial pH variations within a groundnut field may increase the tendency of the pods to be at various stages of physiological maturity at harvest, in addition to variations caused by the tendency of groundnut to be indeterminate in growth habit. More mature seeds at the time of harvest can be expected at higher pH levels.

Experiment 6: Interaction effects of pH and Ca on pod development

Pod formation was observed in all treatment combinations of pH and Ca levels tested (Table 5.4). Approximately 58% of the gynophores cultured in treatment combinations with pH 3.5 produced normal pods, compared to 94% in combinations with pH 5.0 or 6.5. At pH 3.5 and 5.0, increases in solution Ca concentration significantly improved pod production, whereas at pH 6.5 the improvements were not significant, indicating that Ca has an ability to counteract the injurious effects of low pH to groundnut pod growth. In this respect, the alleviation of Ca on injurious effects of low pH on pod growth is similar to the alleviation it has on injurious effects of low pH on root growth (Sanzonowicz *et al.,* 1998). The lack of significant effect of Ca concentrations on pod production at pH 6.5 is also probably an indication of better Ca availability at this pH level, which would result in lower Ca concentrations being adequate for normal pod growth. Once the amount of Ca needed to satisfy the needs of the actively growing meristematic tissues of the pods has been absorbed, the excess absorbed Ca is precipitated within the tissues as insoluble Ca oxalate (Tisdale & Nelson, 1975).

Solution pH had a significant effect on the time taken from submergence of the gynophore to initial expansion of the basal seed compartment (Table 5.4). Generally, the initial expansion of the basal seed compartment was significantly delayed at pH 3.5 compared with pH 5.0 and 6.5 irrespective of the Ca concentration in the solution. On average, the initial expansion of the basal seed compartment at pH 3.5 was observed approximately 11 days after submergence, whereas pod expansion became visible at 6 and 7 days after submergence at pH 5.0 and 6.5 respectively. Generally, increasing the solution Ca concentration from 500 to 2000 :M had little effect on time taken to initial pod expansion. Zharare *et al*. (1998) made similar observations with a number of groundnut lines grown in solution Ca concentrations ranging from 0 to 2500 :M.

The effects of pH and Ca concentrations on time taken to initial expansion of the apical seed compartment were significant, with faster pod expansion being observed at the higher pH and Ca levels (Table 5.4). The effect was more marked for pH than for Ca. When averaged across Ca levels, expansion of the apical seed compartment commenced 9 days after the onset of the basal seed compartment expansion at pH 3.5, and 5 days at pH 5.0 and 6.5. Significant interaction effects between pH and Ca concentrations on time taken to expansion of the apical seed

compartment were observed, showing a greater influence of Ca at low pH. Again this observation confirmed that Ca alleviates the injurious effects of low pH on pod development.

Table 5.4 Pod formations and time to visible pod expansion of groundnut *cv* Jesa cultured in nutrient solution at different pH and Ca concentration levels

PH	Ca level (µM)			
	500	**1000**	**2000**	**Mean**
	Days to initial pod expansion of basal seed compartment			
3.5	11.5	12.08	11.07	11.55
5.0	6.58	6.35	6.92	6.62
6.5	6.33	6.25	5.67	6.08
Mean	**8.14**	**8.23**	**7.89**	**8.08**
$LSD_{(0.05)}$ pH = 0.79	**Ca = Non Significant**		**pH x Ca = Non Significant**	
	Days to initial pod expansion of apical seed compartment			
3.5	10,0	9.92	8.50	11.55
5.0	5.34	5.25	4.87	5.15
6.5	5.00	5.00	4.70	4.90
Mean	**6.78**	**6.72**	**6.02**	**7.2**
$LSD_{(0.05)}$ pH = 0.23	**Ca = 0.23**		**pH x Ca = 0.47**	
	% Cultured gynophores that produced pods			
3.5	52.2	58.7	64.0	58.3
5.0	91.3	93.3	96.7	93.9
6.5	95.0	93.5	93.3	93.9
Mean	**79.5**	**81.8**	**84.7**	**82.0**
$LSD_{(0.05)}$ pH = 1.84	**Ca = 1.84**		**pH x Ca = 2.25**	

**Data are means of three replicates

Kernel Ca concentration

An analysis for Ca concentration of the kernels obtained at the different pH and Ca concentrations showed substantial increases in Ca concentration as the pH was increased from pH 4.0 to 7.0 (Table 5.5). This can possibly be attributed to the reduction in the H^+ concentration at higher pH levels, which would result in less ability of H^+ to inhibit Ca uptake. Increasing the Ca levels in the solution from 500 to 2000 µM substantially increased kernel Ca concentrations. In groundnut, the kernel Ca concentration range 0.04 to 0.08 % is considered sufficient for proper

kernel development (Gascho & Davis, 1994). Thus in the present study, the Ca levels in the kernels (Table 5.5) were adequate in all treatments except the pH 4.0 treatment, and the pH 3.5 at 500 μM Ca treatment. In comparison, kernels produced in sand had a much higher Ca concentration than those produced in solution at a similar pH. This can be ascribed to differences in pod surface area and pod volume, which are important factors influencing Ca absorption by the seed (Boote *et al.,* 1982). Although the pods grown in nutrient solution were just as morphologically normal as those grown in sand, the latter pods were larger, probably because they had a natural mechanical stimulus (contact with soil) for better growth, compared to aeration as the stimulus in nutrient solution. Smal *et al.* (1989) observed increased Ca uptake in pods with higher pod surface area.

Table 5.5 Effect of solution pH and Ca concentration on Ca content of groundnut kernels

Solution pH	**Solution Ca**			
	500	**1000**	**2000**	**Mean**
	Kernel Ca concentration (%)			
4.0	0.03			
5.0	0.05			
6.0	0.07			
7.0	0.08			
3.5	0.03	0.06	0.07	**0.05**
5.0	0.04	0.09	0.12	**0.08**
6.5	0.06	0.10	0.09	**0.08**
6.2 (sand)	0.18			

The pod fresh and dry mass was significantly influenced by solution pH, but little affected by solution Ca concentration (Figure 5.13). The average pod fresh mass at pH 3.5 was 1.52 g pod^{-1}, and was increased to 2.98 and to 4.05 g pod^{-1} at pH 5.0 and pH 6.5 respectively. The pod fresh mass at pH 3.5 was reduced from 2.04 g pod^{-1} at 2000 :*M* Ca to 0.77 g pod^{-1} at 500 :*M* Ca. At pH 6.5 the effect of Ca concentration was much less, indicating that higher levels of solution Ca counteracted the negative effects of low solution pH on pod formation. Similar reductions in pod

dry mass were observed at the same pH level. Differences in pod fresh and dry mass at the different Ca concentrations were of a lesser magnitude at pH 5.0 and 6.5 compared to those observed at pH 3.5. In experiments on effects of solution Ca on concentration in the podding environment on pod dry mass Zharare (1997; 1998) observed significant depressions in dry mass at 0 and at 2500 ΦM Ca, with maximum yield observed at solution Ca ≤ 100 ΦM.

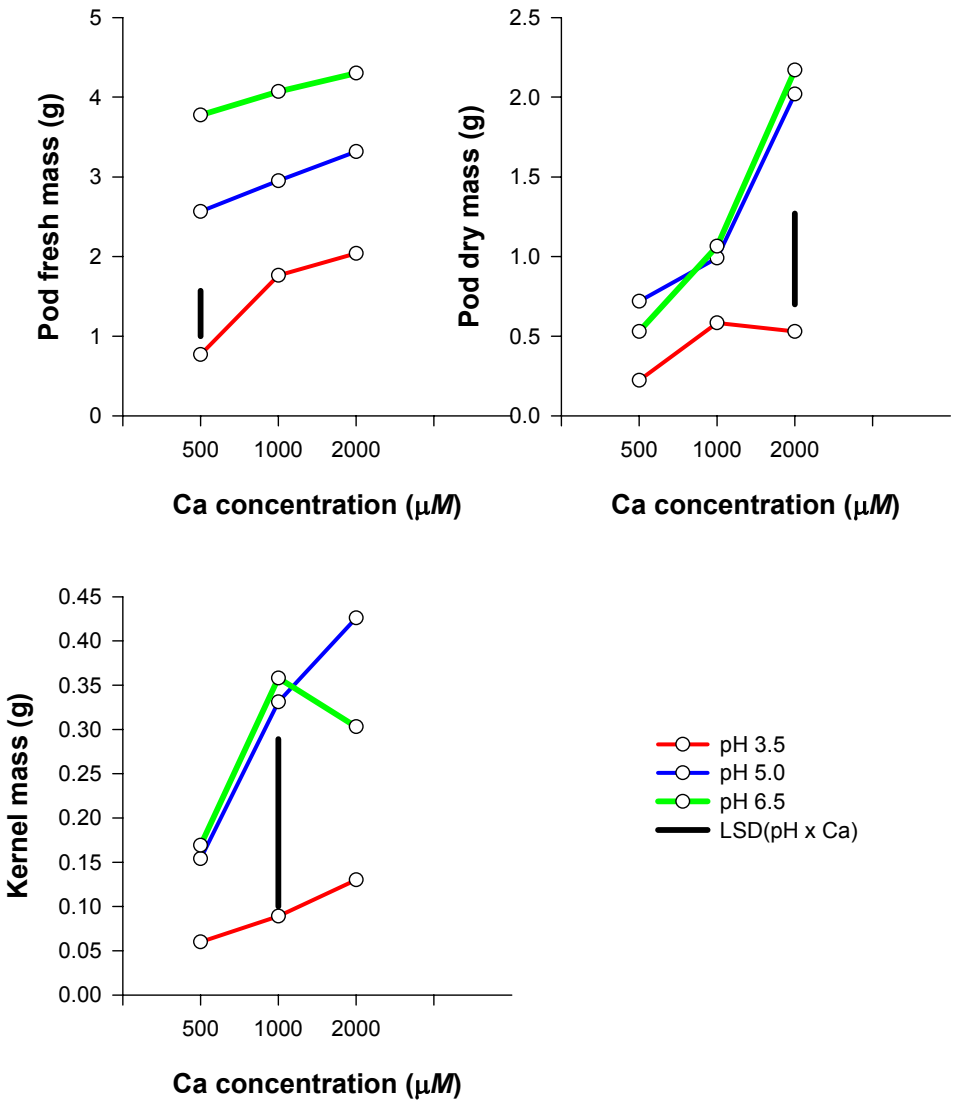


Figure 5.13 Pod and kernel mass of groundnut *cv* Falcon cultured in nutrient solution at different pH and Ca levels

While the impact of increasing the solution Ca concentrations on pod mass was greater at pH 3.5 than at pH 5.0 or 6.5, there was a tendency for pod mass to increase with increase in solution Ca concentrations at all pH levels. Smal *et al.,* (1989) similarly observed increases in pod dry mass of a runner type as the Ca level in the pod zone was increased from 25 to 1875 μ*M*. In the present study, the largest increases in pod mass due to increases in Ca concentrations were observed at pH 3.5 where increasing the Ca concentrations from 500 to 1000 μ*M* increased fresh mass by 130%, and dry mass by 167%. The interaction effects of solution pH and Ca concentration on pod mass showed significant effects at pH 3.5, but a tendency for pod mass to increase with increase in solution Ca concentrations at pH 5.0 and 6.5.

Average kernel weight at pH 3.5 was 0.10 g kernel^{-1}, and was increased to 0.30 g kernel^{-1} at pH 5.0, and to 0.28 g kernel^{-1} at pH 6.5 (Figure 5.13). The kernel weight was highest at pH 5.0, and ranged from 0.15 g kernel^{-1} with 500 μ*M* Ca to 0.43 g kernel^{-1} at 2000 μ*M* Ca. At pH 3.5 kernel weight ranged from 0.08 g kernel^{-1} at 500 μ*M* Ca to 0.13 g plant^{-1} at 2000 μ*M* Ca. Kernel weight responses at pH 6.5 were less than those observed at pH 5.0. The observed pH x Ca interaction showed that the effects of Ca concentration on kernel weight were largest at the intermediate pH level, and diminished at pH 3.5 or 6.5.

Reasons for poor productivity of legumes on acid soils include failure to nodulate as pH decreases (Andrew, 1976; Munns, 1978; Franco & Munns, 1982), reduced nodule function (Franco & Munns, 1982), or limited plant growth (Franco & Munns, 1982). Since none of these factors were observed in my experiment (due to adequate nutrient supply at optimum pH in the root zone), the low yields observed at the low pH levels can be ascribed to the detrimental effects of low pH *per se* on nutrient uptake and growth. According to Kidd & Proctor (2001), plants growing in very acid soils appear to be faced firstly with toxic H^+ ion concentrations before they encounter other unfavourable factors (toxic concentrations of Al and Mn or deficiencies of N, P and Ca), thus supporting the premise that the direct toxicity of the H^+ ion concentration is the proximal cause of the poor growth of non-tolerant plants on acid soils. The low yields at pH 3.5 compared to pH 5.0 or 6.3 imply that the high H^+ activities were toxic irrespective of Ca concentration, in other words, the ameliorating effect of Ca at low pH was limited. Shamsuddin *et al.,* (1992) found little evidence of an ameliorative Ca effect on groundnut nodulation and growth in the presence of toxic concentrations of Al.

As in Experiment 5, the tufts of hair covered with mucilage showed on the surfaces of gynophores cultured at all pH levels and all Ca concentrations, and they continued to show until the pods were harvested five weeks later. Septate and non-septate hairs were observed on gynophore and pod surfaces at all pH and Ca concentrations, but sparse cover of hairs was observed at low pH and Ca concentrations while pods formed at higher pH and Ca levels generally had dense cover of hairs. The ability to form hairs on gynophore surfaces even at low pH and Ca levels could be viewed as plant adaptation to low pH and Ca, and coupled with corresponding root hair formation, could substantially increase the tolerance of groundnut to low pH and Ca.

Microscopic examinations of the excised seeds showed that normal embryos were formed even at pH 3.5 at the lowest Ca concentration (Figure 5.14). However, plumule development was much improved with 1000 μM Ca at pH 5.0 or 6.5 compared to pH 3.5. The cotyledons were not affected by pH or Ca concentration. Microscopic and histological studies by Harris & Brolman (1966) on comparison of calcium and boron deficiencies of groundnut showed that boron deficiency affected the inside of cotyledons and sometimes caused the tips of the plumules to be small and pointed, whereas Ca deficiency affected mainly the vascular system at the base of the plumules.

Little information is available on the effect of Ca on pod growth and maturity of groundnut. However, accelerated growth can be expected at higher Ca concentrations, since Ca^{2+} ions play an important role in cell growth (Bush, 1995). The importance of Ca in plant nutrition stems from its role in membrane stability and the maintenance of cell integrity (Epstein, 1972). With Ca deficiency the membranes become leaky and solutes are lost from the cytoplasm. This means that at low pH levels where Ca^{2+} is displaced by H^{+} (Kinraide *et al.,* 1994), retarded pod growth could be expected. There is a decline in Ca influx in fruits during development because of an increase in solute influx through the phloem, a decline in the rate of cell division and the formation of new binding sites for Ca, and a change in volume/surface area (Kirkby & Pilbeam, 1984). All these factors would be expected to influence pod growth.

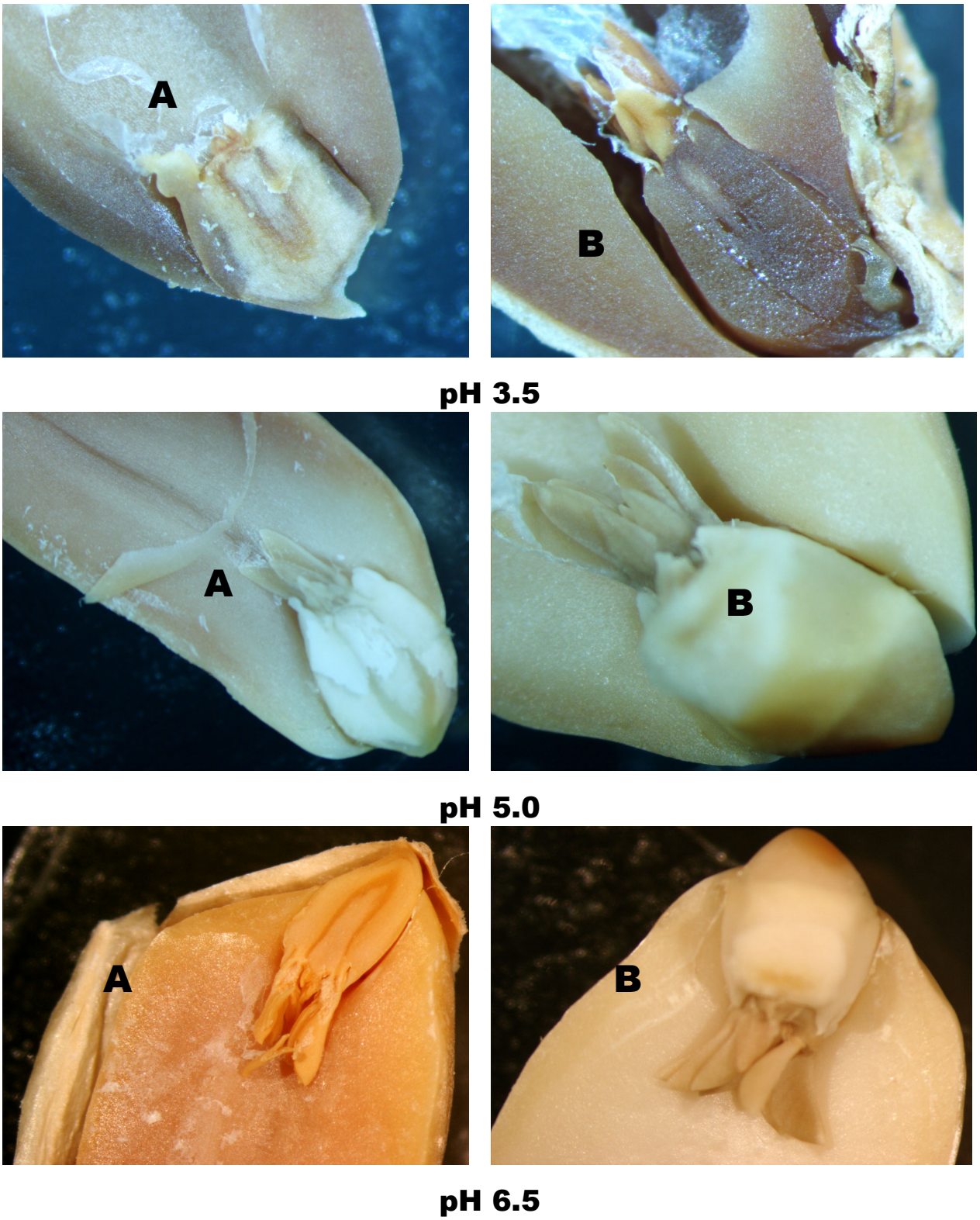


Figure 5. 14 Photomicrographs of cotyledons and embryos produced at different pH levels with 500 µM Ca (A) and 1000 µM Ca (B)

5.4 CONCLUSIONS

5.4.1 GERMINATION, SEEDLING SURVIVAL AND EARLY GROWTH

The results of this study indicate that low pH *per se* does not have a major impact on the germination of groundnut seed, but significantly influences the seedling survival and early growth. The germination of groundnut seed was tolerant of low solution pH; given that even at pH 3.0 a germination percentage of 86% was attained, and that increasing the pH in the range 4.0 to 6.0 had no appreciable effect on germination.

The adverse effects of low pH on germination and seedling survival were more pronounced in the absence of Ca, and became progressively less as the solution Ca concentration increased. Seedling survival was more sensitive to the effects of pH than seed germination, and both parameters were improved as the Ca concentration and pH values were increased. Groundnut seedlings survived best in the pH range 5.0 - 6.0. Seedling growth (root and shoot dry mass, root length and root surface area) also improved with increasing Ca concentrations in the solution. The combination of low Ca and low pH severely retarded lateral root formation. These results imply that early growth of groundnut can be improved in strongly acid soils if adequate Ca is made available to the germinating seed.

5.4.2 REPRODUCTIVE GROWTH

This work has shown that low pH *per se* has a significant effect on pod formation, yield and quality of groundnut. Pod initiation and expansion were highly sensitive to low solution pH, given that the latter caused significant delays in pod initiation, and resulted in no meaningful pod expansion, with only 12% and 55% of the cultured gynophores developing into pods at pH 3.0 and pH 4.0. Groundnut pod and kernel yields were best in the pH range 5.0 - 6.0, so was kernel quality. At lower pH values the quality of the seed was markedly deteriorated.

Low pH was more deleterious to pod initiation and expansion in the absence of Ca, and the damage was ameliorated by increasing the solution Ca concentration, thus indicating the involvement of a Ca-requiring process in overcoming proton toxicity as observed by Koyama *et*

al., (2001). At high solution pH levels (pH 5.0 and 6.5) Ca concentration had smaller effects on pod initiation, development and dry mass production compared to pH 3.0.

The observed dense pod hair formation and persistence at higher pH levels implies that uptake of Ca and other nutrients by the developing pods might be increased at higher solution pH levels. The persistence of the pod hairs during the crucial pod initiation stage would ensure adequate Ca supply, which would result in normal pod development.

These results support the hypothesis that in addition to Ca deficiency, high H^+ ion concentration *per se* can be a limiting factor for groundnut productivity in acid soils. It also implies that productivity of groundnut can be improved in strongly acid soils if adequate Ca is made available to the developing pods.