Title

Interactions of pod-zone pH and Ca concentrations on reproductive growth of groundnut (Arachis hypogaea L.)

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Introduction

Soil acidity is a major problem in groundnut (*Arachis hypogaea* L.) production. Soils most suitable for groundnut production are those that are light textured/sandy (Henning et al., 1982). Because of their small reservoir of alkaline cations and high leaching potential, the soils are highly prone to acidification (Kamprath and Smyth, 2004; Fageria and Baliga, 2008). In a recent study Murata et. al., (2008) showed that low pod-zone pH adversely affects groundnut fructification. Often, soil acidity is accompanied by Ca deficiency (Fageria and Baliga, 2008), which also adversely affects groundnut fructification (Gascho and Davis, 1994). Thus, both lime and gypsum applications are often required to ensure high groundnut yield (Gascho and Davis, 1994). Poor-resource farmers may not afford application of both lime and gypsum, which necessitates a close examination of the options for managing acid soils for groundnut production.

Lime applications in groundnut have always been assumed to benefit the crop by increasing Ca in the pod-zone in addition to ameliorating pH in the root-zone (Gascho and Davis, 1994). Evidence supplied by Murata et al. (2008) indicates that liming an acid soil that also raises the pH in the pod-zone should enhance pod production. In the root-zone, Ca overcomes injurious effects of low pH on root growth (Lu and Sucoff, 2001**;** Yan et al., 1992) without necessarily increasing the pH of the growth medium. Similarly, application of Ca to the pod-zone on acid soils might be expected to relieve the adverse effects of low soil pH on groundnut fructification. The present study examines whether Ca ameliorates the adverse effect of low pod-zone pH on groundnut pod production.

Materials and Methods

Plant and pod culture

Plants of Spanish groundnut *cv.* '*Faclcon',* were raised in a coir/sand mix contained in rectangular PVC boxes (58 x 48 x17 cm). In each box, there were six plants in two rows spaced 35 cm apart and 10 cm within row. Throughout the experiment, the plants were drip-irrigated with a complete nutrient solution containing (μ*M*): 400 sulphur (S), 250 nitrogen (N), 250 potasium (K), 300 calcium (Ca), 100 magnesium (Mg), 2 phosphorous (P), 10 iron (Fe) [as iron ethylenediaminetetraacetic acid (FeEDTA)], 10 chlorine (Cl), 3 boron (B), 0.25 zinc (Zn), 0.10 manganese (Mn), 0.07 copper (Cu) and 0.02 molybdenum (Mo).

Flowers and gynophores produced close to the base of the plants were removed to encourage flowering higher up the plant for experimental convenience. Ten glass test tubes were buried in the sand around each plant, and gynophores of approximately the same age and which were approximately 5 cm long were individually positioned in 15 ml of a simplified nutrient solution containing 500, 1000 or 2000 μM Ca (added as CaSO4) in factorial combination with solution pH levels 3.5, 5.0 and 6.5. Additional nutrients to S and Ca present in the pod culture solution were 4 μM Fe (as Fe-EDTA) and 0.5 μM Zn. A simplified nutrient solution containing only Ca, S, Fe and Zn was observed to produce normal and healthy pods by Zharare (1997). The pod culture solution was adjusted with 0.1*M* H_2SO_4 to obtain the target treatment pH values. 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 test tubes were loosely covered with aluminium foil to allow adequate aeration but exclude light from the pod culture solution. The pod culture solutions were 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.

Data collection

Time taken to initial pod expansion of the basal and apical seed compartments was recorded, and so were the number of cultured gynophores that produced normal pods. Representative pods were harvested at intervals and their surface tissue checked for hair development by scanning electron microscopy. At 5 weeks after submergence of the gynophores into the nutrient solution, a total of 10 mature pods per treatment were harvested. Three fresh pods were checked for surface hairs by scanning microscopy. Seven pods were dried at 60 ^{O}C until constant weight, and the dry weight determined. Cotyledons of dry seeds from three of the seven dry pods were separated to reveal intact plumule, hypocotyl and radicle attached to one of the cotyledon, which were then examined with a light microscope to assess embryo development. Dry seeds of four replications were digested in a nitric : perchloric acid mixture (5:1 vol/vol) and analysed by an inductively coupled plasma atomic emission spectrometer for tissue Ca concentration.

Results and Discussion

Pod-set and development

Pods formed in all treatment combinations of pH and Ca levels tested (Table 1), but their development was significantly affected by both the pH and Ca concentration of the culture solution. Approximately 58% of the gynophores cultured at pH 3.5 produced normal pods, compared to 94% at pH 5.0 and 6.5. This confirmed observations made by Murata et al. (2008) in which low pH was also shown to adversely affect groundnut pod-set. At pH 3.5 and 5.0, increasing the solution Ca concentration from 500 to 1000 and 2000 µM significantly improved pod production (Table 1). At pH 6.5, there was a tendency for the pod production to decrease at solution Ca concentration > 500 µM though the decreases were not significant. The influence of Ca was thus greater at low pH than at high pH, indicating that external Ca requirements for pod-set are higher at low than at high pH as has been noted for root growth (Lu and Sucoff, 2001; Yan et al., 1992).

Pod expansion in the basal compartment preceded that of the apical seed compartment as described by Zharare et al. (1998) for groundnut lines TMV-2, Chico and CBRR4. Generally, the initial expansion of the basal seed compartment was significantly delayed by 5 and 4 days in gynophores submerged in solution at pH 3.5 compared with pH 5.0 and 6.5, respectively, irrespective of the Ca concentration in the pod culture solution (Table 1). Thus, solution Ca concentration did not affect the time taken to initial expansion of the basal seed compartment as has been reported by Zharare et al. (1998). By contrast, the time taken to initial expansion of the apical seed compartment was significantly affected by both the solution pH and Ca concentration, with faster pod expansion being observed at the higher pH and Ca levels (Table 1). The effect was, however, more marked for pH than for Ca. Significant interaction effects between pH and Ca concentration on time taken to expansion of the apical seed compartment were also observed, showing a greater influence of Ca at low pH. Again, this confirmed that Ca alleviates the injurious effects of low pH on pod development.

Table 1. Pod formation and time to visible pod expansion of groundnut vc Jesa cultured in nutrient solutions at different pH and Ca concentration levels (data are means of three replications).

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
Means	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
Means	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
Means	79.5	81.8	84.7	82.0
$LSD_{(0.05)}$ pH=1.84; Ca=1.84; pH x Ca=2.25				

The pod dry mass was significantly depressed at pH 3.5 compared with pH 5.0 and 6.5 (Figure 1A). Increasing the solution pH from 5.0 to 6.5 did not have significant effects on dry pod mass. The effects of Ca supply on dry pod mass were small and non significant at pH 3.5, whereas pod dry mass was positively affected at pH 5.0 and 6.5 by increasing solution Ca.

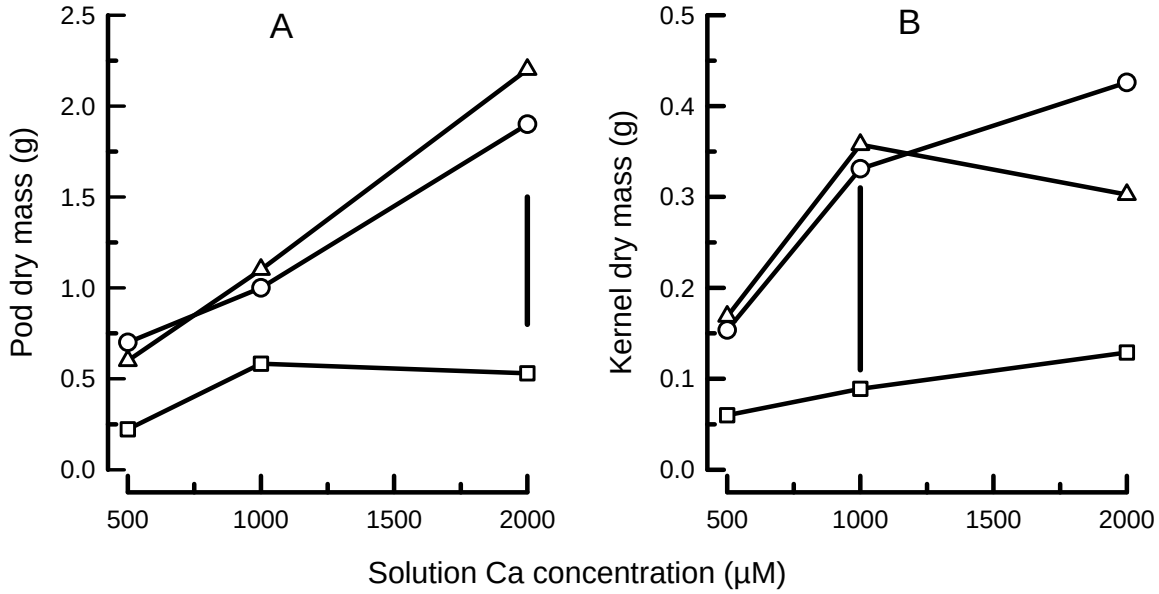

Figure 1. Effect of solution Ca concentration on pod (A) and seed (B) dry mass of groundnut *cv.* Falcon grown at solution pH 3.5 (□), 5.0 (○) and 6.5 (Δ). (Data points are means of three replications.)

As was pod mass, also dry seed mass was severely depressed at pH 3.5 (mean 0.09 g) compared with pH 5.0 (mean 0.30 g) and pH 6.5 (mean 0.28 g) which again emphasized the deleterious effects of low pH on groundnut fruit growth. At pH 3.5 there was a tendency for the seed mass to increase with increasing solutions Ca concentration (Figure 1B). At pH 5.0 and 6.5, increasing the solution Ca concentration to 1000 µM significantly enhanced kernel dry weight. Further increasing the Ca supply tended to further increase the kernel dry mass at pH 5.0 but to decrease it at pH 6.5. Overall, the observed pH x Ca interaction showed that the effects of Ca concentration on seed weight were largest at the intermediate pH level (5.0), and diminished at pH 3.5 or 6.5. The smaller effects of Ca on seed growth at pH 3.5 compared to pH 5.0 or 6.5 imply that the ameliorating effect of Ca at low pH was limited.

The reduction in seed growth at both high pH and Ca levels observed at pH 6.5 x 2000 µM in the present study and also by Murata et al. (2008) and Zharare et al., (1998) may involve Ca-induced and/or pH-induced nutrient deficiencies. Zharare (1997) noted strong antagonism of pod-zone Ca on Mg, Mn, Zn and Fe concentrations in seeds and pod shells which increased as the solution Ca concentration increased. Also, high soil pH is known to adversely affect uptake of micronutrients by plants (Fageria et al., 2002; Fageria and Baligar, 2008). A combination of high pH and high Ca concentration in both the root and pod-zone could therefore be expected to severely restrict pod uptake of micronutrients with deleterious effects on pod growth. For example, antagonism on Zn uptake from the pod-zone by high pod-zone pH or Ca may impair groundnut pod and seed development (Zharare et al., 1993).

Ca concentration in seed tissue

Tissue Ca concentrations in the seeds were significantly lower at pH 3.5 than at pH 5.0 and 6.5, and the difference was greater at 1000 and 2000 µM Ca than it was at 500 µM Ca (Figure 2). At

all solution pH, the response of tissue Ca concentration in the seeds was greater when the solution increased from 500 to 1000 µM Ca than when the solution Ca concentration was increased from 1000 to 2000 µM Ca (Figure 2). Except at pH 3.5, the Ca concentrations in the seed tissue were above the range 0.38-0.041% reported by Adams et al. (1993) to be adequate for groundnut germination. Thus the results question the validity of this optimum range since growth of pods was negatively affected in this study even at higher Ca concentrations (Figure 1).

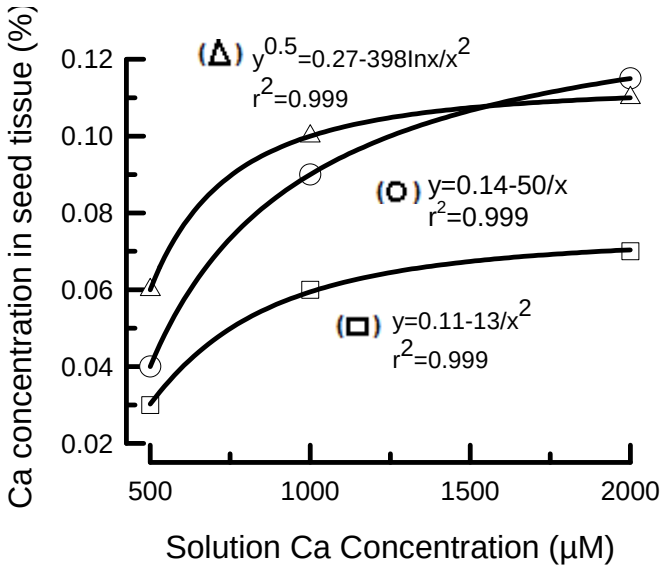

Figure 2. Relationships between solution Ca concentration and tissue Ca concentration in seeds of groundnut grown in pod culture solution at solution pH 3,5 (□), 5.0 (○) and 6.5 (Δ). (Data points are means of three replications.)

Pod hair development

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 described by Zharare et al. (1993) were observed on gynophores and pod surfaces at all pH and Ca concentrations, but were sparse at low pH and Ca concentrations (not shown). The ability to form hairs on gynophores and pod surfaces even at low pH and Ca levels could be viewed as plant adaptation to low pH and Ca, and reflect the tolerance of groundnut of low pH and low Ca.

Embryo development

Microscopic examinations of excised seeds showed that embryos were formed even at pH 3.5, the lowest Ca concentration. However, plumules in the mature seeds were not well developed in treatment combinations of pH 3.5 and 500 µM compared with higher solution Ca concentrations and pH (Figure 3). The poor plumule development at pH 3.5 x 500 µM Ca coincided with tissue Ca concentrations in seeds (Figure 1A) that were below the levels (0.38-0.041%) considered adequate for successful germination by Adams et al. (1993). However, poor plumule

development also at higher Ca concentrations (Figure 3) support the questioning of the validity of the deficiency threshold.

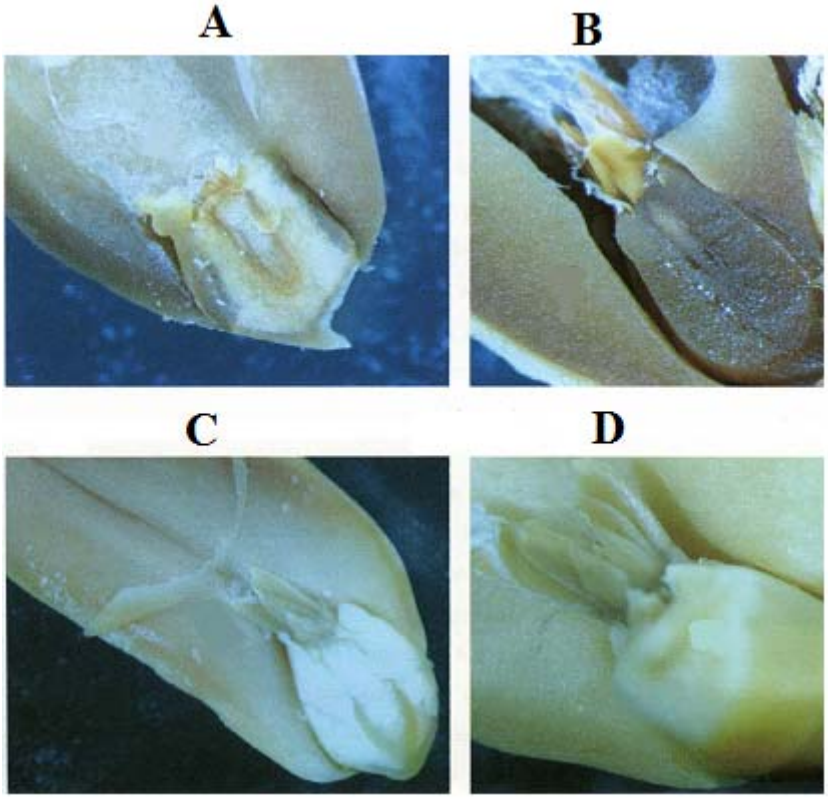

Figure 3. Micrograph of cotyledons and embryos produced at pH 3.5, 500 µM Ca (A) pH 3.5, 1000 µM Ca (B), pH 5.0, 500 µM Ca (C) and pH 5.0, 1000 µM Ca (D) . Note the absence of a plumule in (A) and the poorly developed plumules in (B) and (C) compared with (D).

Conclusion

Low pH severely affected pod-set, pod mass and seed mass. These parameters were improved by increasing the Ca concentration in the culture solution, but the improvements were much smaller that those caused by increasing the pH of the solution. It was concluded that liming the pod-zone to moderate pH may be a better option than application of gypsum on very acid soils. The results have important applications for low input agriculture where farmers may not afford to apply both lime and gypsum for groundnut production in acid soils.

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