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**Title**

Some observations on reproductive growth of groundnut (*Arachis hypogaea* L.) in simplified nutrient solutions

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**Authors**

Zharare, Godfrey Elijah  
Asher, Colin J  
Blamey, Pax F. C.

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## Introduction

Groundnut is one of the few plant species in which a fertilized ovary of an aerial flower must be buried in the soil for the fruit (pod) to grow further and mature (Brennan, 1969). After flowering and fertilization above ground, further embryo development and fruit expansion are suspended while an intercalary meristem at the base of the ovary produces a stem-like gynophore (Brennan, 1969). The gynophore, carrying the ovary at its tip, bends and elongates downwards to penetrate the soil. Once the ovary is sufficiently buried (5 -10 cm deep), embryo development is resumed and fruit expansion occurs. In its subterranean position, the pod does not transpire and so does not receive xylem sap from the roots. Consequently, the developing pod and buried portions of the gynophore are forced to absorb phloem-immobile mineral nutrients directly from the pod-zone (Skelton and Shear, 1971), but they also have an option of importing phloem-mobile mineral nutrients from the roots in addition to importing sugars, and via the phloem. Currently, the nutrient absorption dynamics of the reproductive structures of groundnut from the pod-zone are not known. Gaining this knowledge would greatly facilitate the management of the mineral nutrition of the crop.

Traditionally, detailed studies on nutrient absorption by plants have involved exercised roots in association with short-term absorption periods from a simplified nutrient solution containing Ca and the nutrient of interest. The use of a simplified nutrient solution is desirable as it minimizes complications associated with interacting nutrients in complex nutrient solution, but deficiencies of omitted nutrients in a simplified nutrient solution may disrupt the physiological functioning of the absorbing organ (White, 1973). Whilst, this can be mitigated by short uptake period, the data obtained may not represent the typical (White, 1973).

Since gynophores and developing pods do receive phloem-mobile mineral nutrients, it is reasonable to assume that a simplified nutrient solution can be used for conducting long-term nutrient absorption studies with attached gynophores and developing pods without disrupting the physiological functioning of the organs. Consequently, an observational study was conceived to assess if healthy groundnut pods could be produced in a simplified nutrient solution containing only calcium (Ca), sulphur (S), zinc (Zn) and iron (Fe) with the aim of using the solution for detailed long-term studies on absorption of Ca and selected mineral nutrients by attached developing groundnut pods without the complications of interacting nutrients commonly observed with complex nutrient solutions. An inventory of the literature indicated that adequate Ca in the pod environment is necessary for healthy pod development. Zharare et al. (1993) showed that Zn, but not magnesium (Mg) or manganese (Mn) is required also in the pod-zone. Further more, Welch and Norvell (1993) gave evidence indicating that Zn is required for the uptake and retention of several mineral nutrients by roots. Omission of boron (B) from the pod-zone has no adverse consequence on groundnut pod development (Campbell et al. 1975). Other nutrients such as Mg, nitrogen (N), chlorine (Cl), K and sodium (Na) are phloem mobile; hence, their omission from the pod-zone might not affect groundnut pod development adversely. Mobility of iron (Fe) in the phloem is low, hence its inclusion in the simplified nutrient solutions.

## Materials and Methods

Three healthy plants for each of groundnut lines Virginia Bunch 1 (Virginia bunch), TMV-2 (Spanish) and CBRR4 (Valencia) were raised in summer (air temp 29-34°C) singly in 20 L

drums, each with a nutrient solution containing ( $\mu\text{M}$ ); 2500 Ca, 2600 S, 250 K, 250 N, 100 Mg, 7 Na, 5 silicon (Si), 3 B, 4 Fe (as iron ethylenediaminetetraacetic acid (FeEDTA)], 8 P, 0.5 Zn, 0.25 Mn, 0.15 copper (Cu), 0.04 cobalt (Co), 0.02 nickel (Ni) and 0.02 molybdenum (Mo) (ionic strength approx. 9 mm).

Six gynophores per plant (7 to 8 cm long for VB-1 and 4 to 5 cm long for TMV-2 and CBRR4) of each line were immersed at the same time in a darkened, aerated solution containing only ( $\mu\text{M}$ ); 100 Ca, approx. 101 S, 2 Fe and 0.5 Zn, in 250 mL vials. For comparison, an additional set of three gynophores per plant for each line were immersed in the simplified nutrient solution, but without Ca. The solutions in the vials were replaced with fresh solutions every 3 d. The pods were grown for 38 d during which the air and solution temperatures averaged 32 and 29 °C, respectively.

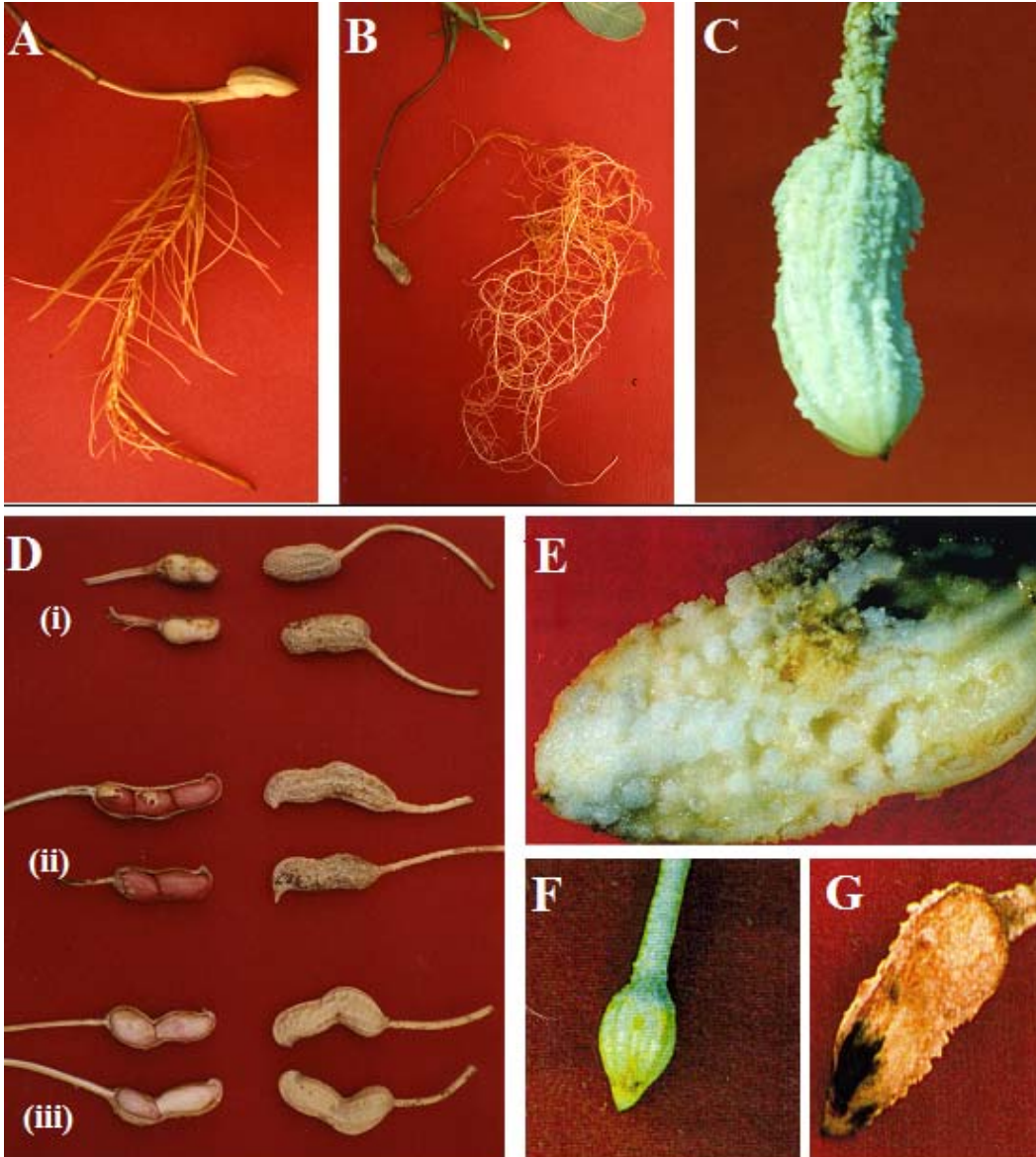
## Results and Discussion

Pod expansion started at approximately 4, 6 and 10 days for TMV-2, CBRR4 and VB-1, respectively after submergence of the gynophores in the culture solution irrespective of the solutions contained or did not contain Ca. The number of days to initial pod expansion were similar to the mean number of days to initial pod expansion observed for the same lines grown in summer in a complete nutrient solution containing 100  $\mu\text{M}$  Ca (Zharare et al, 1998). Surprisingly, root development was observed at approximately 2.5-3 cm above the point of pod attachment to the gynophore (Figures 1A; 1B) in three of CBRR4 gynophore cultured in the simplified nutrient solution containing Ca. The gynophores that developed roots were on two of the three CBRR4 plants that were grown. Formation of roots on groundnut gynophore has not been observed in the cultivated groundnut before, and neither is there information in the literature indicating that they do form freely on gynophores of the wild *Arachis* species. The roots formed by CBRR4 gynophores were not adventitious as might be expected if they were induced by wounding (Shen et al. 1995), but had a structure that is typical of a tap root system, complete with side branches (Figure 1A). Also, the fact that they occurred in three gynophore cultures suggest that the phenomenon might not have been a fluke accident. The roots were formed at approximately similar distances from the point of pod attachment to the gynophore and on two of the three CBRR4 plants which strongly support that their formation was not an accident.

The factors that caused roots to form in some of CBRR4 gynophores cannot be deduced from this study, but the ability of gynophores to form roots might be genetic, since TMV-2 and VB-1 gynophores did not form roots. The production of roots by attached gynophores in susceptible groundnut cultivars in a simplified nutrient solution might be useful in studying long-term nutrient absorption dynamics of roots. Because, they are not the principle nutrient absorbing organs of the plant, and they can receive phloem mobile mineral nutrients, there is a possibility that they can be used for long-term mineral nutrient absorptions studies in simplified nutrient solutions without disrupting their physiological functions.

The pods and kernels of CBRR4 that developed on these gynophores that formed roots were normal and apparently healthy (Figure 1A; 1B), and so were the rest of CBRR4 pods and those of TMV-2 and VB-1 which formed on gynophores cultured in solution containing Ca (Figures 1C; 1D). Whilst all lines produced pods on all gynophores cultured in the solution without Ca, pod growth was severely restricted, especially in VB-1 (Figure 1F) whose pods rot

and did not reaching maturity. In addition, the pods showed symptoms that could be attributed to Ca deficiency. The symptoms started as brown colouration of septate pod hairs or development of soft, yellow-green patches on pod surface (Figures 1E; 1F). These later turned brown and eventually black, giving the pod a mottled surface (Figure 1G).



*Photomicrographs showing root development on attached gynophores of CBRR4 (A; B), normal and healthy immature CBRR4 pod (C), mature pods and kernels (D) of (i) TMV-2, (ii) CBRR4 and (iii) VB-1 cultured in solution containing 100  $\mu\text{M}$  Ca, 2  $\mu\text{M}$  Fe and 0.5  $\mu\text{M}$  Zn; immature TV-2 pod (E), immature VB-1 pod (F) and mature CB CBRR4 pod (G) produced in solution containing 2  $\mu\text{M}$  Fe and 0.5  $\mu\text{M}$ . Note the formation of typical tap root systems in (A) well developed and healthy roots in (B); abundant and healthy white septate hairs in (C); production of normal and healthy mature pods and kernels in D; and calcium deficiency showing as browning of hairs in (E), yellowish patches in (F) and blackening of the apical seed compartment in (G).*

Visual inspection indicated that the cultivars differed in the density of septate pod hairs. These were most dense in CBBR4 and least abundant in VB-1. On VB-1, the hairs were confined to the reticulation ridges on the pods. The differences in formation of pod hairs between the cultivars strongly suggest genetic influence and may have important implications in the ability of the cultivars to absorb Ca from the pod-zone, especially under deficiency conditions.

In conclusion, the nutrient solution containing 100  $\mu\text{M}$  Ca, 2  $\mu\text{M}$  Fe and 0.5  $\mu\text{M}$  Zn supported production of normal and apparently healthy groundnut pods as well as healthy roots in those gynophores cultures of CBBR4 that produced them. It must however be cautioned that the root-zone must be able to supply the rest of the nutrients in adequate amounts.

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