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Full Length Research Paper

Some observations on the culture of attached groundnut (*Arachis hypogaea* L.) gynophores in simplified nutrient solutions

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Attached groundnut gynophores of Virginia line Virginia bunch strain 1 (VB-1), Spanish line TMV-3 and Valencia line CBBR4 were cultured singly in aerated, simplified nutrient solutions consisting of (2 µM iron (Fe) and 0.5 µM zinc (Zn) with or without 100 µM calcium (Ca). In all lines, normal and apparently healthy pods were produced in solutions containing Ca, whereas, pod growth was severely restricted in solutions without Ca. Three of CBRR4 gynophores cultured in solution containing Ca formed healthy tap roots. It was concluded that a simplified solution containing only Ca, Zn and Fe could support healthy groundnut pod growth.

Key words: *Arachis hypogeae*, groundnut gynophore, pod development, root formation, solution culture.

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; Moctezuma, 1999). 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 resumes and fruit expansion occurs. In its subterranean position, the pod does not transpire and does not receive xylem sap from the roots. Consequently, the developing pod and buried portions of the gynophore must absorb phloem-immobile mineral nutrients directly from the pod-zone (Skelton and Shear, 1971; Zharare et al, 2009), while phloem-mobile mineral nutrients from the roots may be imported, along with sugars, 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 excised 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 interactions among nutrients in complex nutrient solutions, but deficiencies of omitted nutrients in a simplified nutrient solution may disrupt the physiological functioning of the absorbing organ (White, 1973). While this can be mitigated by a short uptake period, the data obtained may not represent typical whole-plant behaviour (White, 1973; Lucash et al., 2007).

Since gynophores and developing pods are able to receive phloem-mobile mineral nutrients absorbed by roots, it is reasonable to suppose that a simplified nutrient solution could be used for conducting long-term nutrient absorption studies with attached gynophores and developing pods without disrupting the physiological functioning of these organs. Consequently, an observational study was undertaken to determine 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. A survey of the literature indicated that adequate Ca in the

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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. Furthermore, Welch and Norvell (1993) produced evidence indicating that Zn is required for the uptake and retention of several mineral nutrients by roots. Iron was included in the solution because of its low import via the phloem by legume fruits (Hocking and Pate, 1977) and is accumulated at considerably higher concentrations than other micronutrients in groundnut pods (Zharare, 1993). Sulphur was used as the counter-ion to the cations. Boron was excluded on the basis of the evidence of Campbell et al. (1975) that omission of boron (B) from the pod zone had no adverse consequence on groundnut pod development. Other nutrients such as Mg, nitrogen (N), chlorine (Cl), and potassium (K) are highly phloem mobile; hence, their omission from the pod-zone was not expected to affect groundnut pod development adversely. Micronutrients copper (Cu), manganese (Mn) and molybdenum (Mo), all of which have variable mobility in the phloem (Rengel, 1999), were excluded on the assumption that adequate amounts would be supplied from the roots.

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 singly in 20 L drums, each containing a nutrient solution consisting of (µM); 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 Cu, 0.04 cobalt (Co), 0.02 nickel (Ni) and 0.02 Mo (ionic strength approx. 9 mM).

Six gynophores per plant (7 - 8 cm long for VB-1 and 4 - 5 cm long for TMV-2 and CBRR4) of each line were immersed on the same day in darkened, aerated solutions containing only (µM); 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 was immersed in the simplified nutrient solution, but without Ca. The solutions in the vials were replaced with fresh solution every 3 days. The pods were grown for 38 days, 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 whether or not the solutions contained Ca. The number of days to initial pod expansion was similar to those observed for the same lines grown in summer in a complete nutrient solution containing 100 µM 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 and B) in three of CBRR4 gynophores 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. Also, while roots may naturally and freely form on organs of perennial plants, similar root formation on organs of annual plants such as groundnut is rare. The roots formed by CBRR4 gynophores were not adventitious as might be expected if they had been induced by wounding (Shen et al., 1995), but had a structure typical of a tap root system, complete with side branches (Figure 1A). The fact that they occurred in three gynophore cultures suggest that their production was not accidental. 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 further supported that their formation was not accidental.

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 they did not form on TMV-2 and VB-1 gynophores. 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. Since they are not the principal nutrient absorbing organs of the plant, and they can receive phloem mobile mineral nutrients, there is a possibility that they could be used for long-term mineral nutrient absorption studies in simplified nutrient solutions without disrupting their physiological functions. The roots looked healthy, suggesting that they obtained adequate nutrients which were not present in the pod-zone, from the root-zone via long distance transport in the plant. In addition, the pods and kernels of CBRR4 plants that developed on the gynophores that formed roots were normal and apparently healthy (Figures 1A and B), and so were the rest of CBBR4 pods and kernels, and those of TMV-2 and VB-1 which formed on gynophores cultured in solution containing Ca (Figures 1C and D).

While 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 developing pods rotted and could not reach maturity. Furthermore, the pods grown in solutions lacking Ca showed symptoms consistent with Ca deficiency, which may include brown colouration of pod hairs or development of soft, yellow-green patches on pod surface (Figures 1E and F). These later turned brown and eventually black, giving the pod a mottled surface (Figure 1G).

Visual inspection indicated that the cultivars differed in the density of septate pod hairs. The pod hairs were most dense in CBBR4 and least abandon in VB-1. On VB-1, the hairs were confined to the reticulation ridges on the pods. The differences in the density of pod hairs between

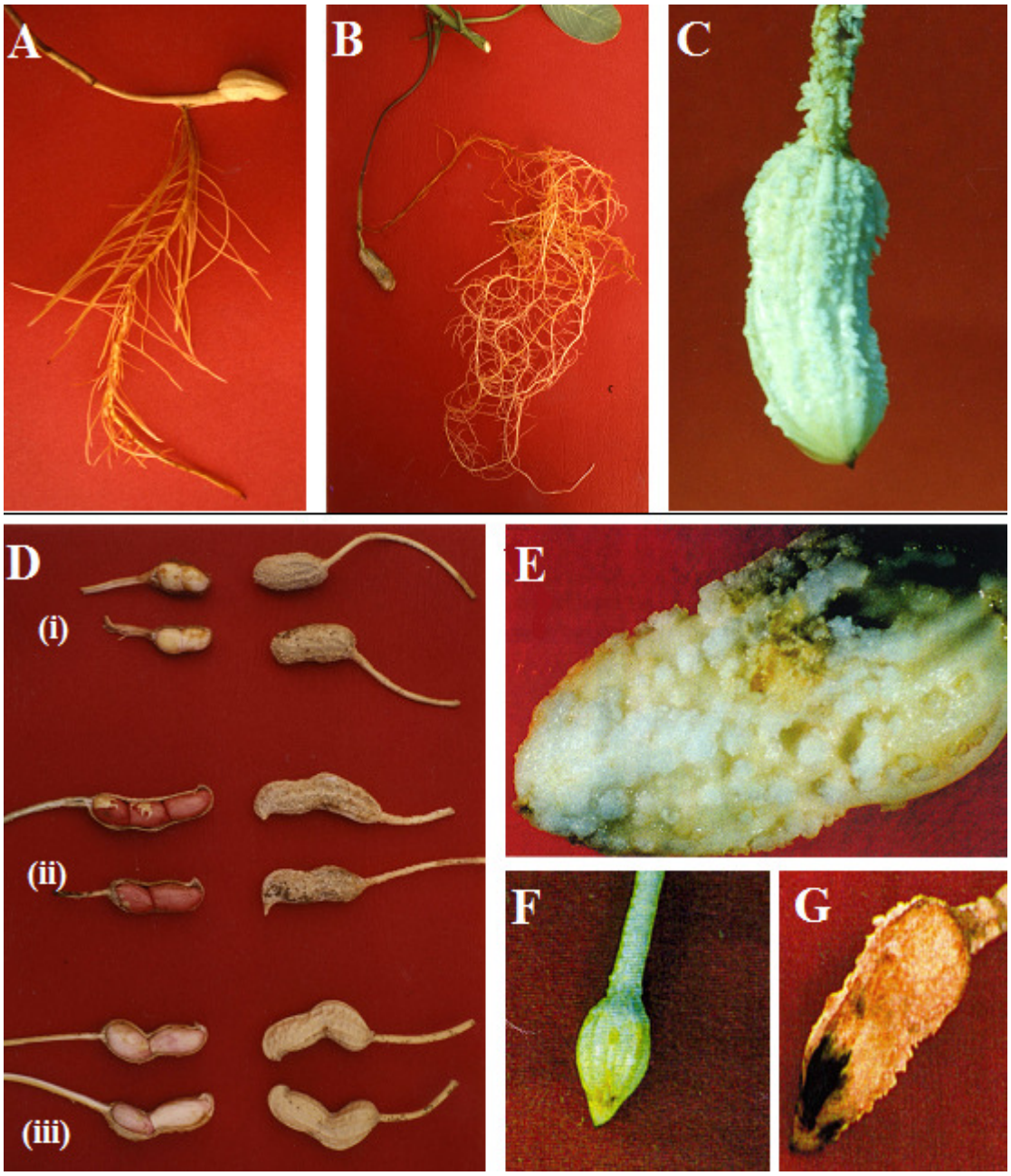

Figure 1. Photographs showing root development on attached gynophores of CBRR4 (A; B), normal and healthy immature CBRR4 pod (C), mature pods and kernels (D) of (i) TMV-2, (ii) CBRR4) and (iii) VB-1 cultured in solution containing 100 µM Ca, 2 µM Fe and 0.5 µM Zn; immature TV-2 pod (E), immature VB-1 pod (F) and mature CB CBRR4 pod (G) produced in solutions containing no Ca. Note the formation of a typical tap root system in A; well developed and healthy roots in A and B; abundant and healthy white septate hairs in C; production of normal and healthy mature pods and kernels in D; and Ca deficiency showing as browning of hairs in E; yellowish patches in F; and blackening of the apical seed compartment in G.

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 µM Ca, 2 µM Fe and 0.5 µM Zn supported production of normal and apparently healthy groundnut pods in all three groundnut cultivars tested as well as healthy roots in those gynophores cultures of CBRR4 that produced them. It must however be cautioned that the root-zone must be able to supply the remainder of the nutrients in adequate amounts.

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