Pertanika 9(2), 135 – 146 (1986)

Effects of Solution, Soil and Sand Cultures on Nodulation and Growth of Phasey Bean

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Key words: Nitrogen-free nutrient solution; nodulation; nitrogen and dry matter accumulation.

ABSTRAK

Pokok kacang phasey (Macroptilium lathyroides *cv. Murray) ditanam di dalam kultur larutan nutrien tanpa nitrogen, tanah dan pasir dalam rumah kaca. Pembintilan, tumpukan bahan kering dalam bahagian-bahagian pokok dan hasil biji ditentukan. Nitrogen simbiotik yang terbahagi di bahagian-bahagian pokok di peringkat tampang dan mengawan ditentukan juga. Di dalam semua media kultur bilangan dan saiz bintil meningkat dengan peningkatan umur pokok, tetapi kadar peningkatan ini pada umumnya lebih cepat dalam kultur larutan daripada kultur-kultur lain. Dalam kultur pasir, berat kering bintil sepokok dan sebintil serta tumbesaran pokok adalah terjejas. Walaupun pemanjangan akar tunjang sentiasa lebih baik dalam kultur larutan daripada kultur tanah atau pasir, perkembangan daun dan tumpukan bahan kering dalam akar dan batang ditingkatkan oleh kultur larutan hanya di peringkat berbunga dan berbuah sahaja. Hasil biji meningkat dengan ketara dalam kultur larutan, satu kesan yang berkaitan dengan pengikatan nitrogen simbiotik yang tinggi. Semasa pertumbuhan tampang kebanyakan nitrogen tertumpuk dalam daun dan batang, tetapi lenggai adalah sink nitrogen yang utama semasa peringkat mengawan. Kebaikan dan kegunaan kultur larutan dalam kajian perkembangan bintil dan koleksi sampel akar untuk asai-asai penurunan asetilena dibincangkan.*

ABSTRACT

Plants of phasey bean (Macroptilium lathyroides *cv. Murray) were grown in nitrogen-free nutrient solution, soil, or sand culture in a naturally-lit glasshouse. Nodulation, dry matter accumulation in plant parts, and seed yields were assessed. Partitioning of symbiotic nitrogen into various plant parts during vegetative and reproductive growth stages was also determined. In all culture media, nodule number and size increased with plant age but the rate of increase was generally greater in solution than in the other cultures. In sand culture, the dry weight per nodule and per plant, and plant growth were significantly suppressed. Although tap root elongation was consistently better in solution than soil or sand culture, leaf development and dry matter accumulation in roots and stems were enhanced by solution culture only during flowering and fruiting stage. Seed yields were significantly increased by solution culture, an effect apparently associated with increased symbiotic nitrogen fixation. During vegetative growth, nitrogen accumulated largely in the leaves and stems but pods were major sinks of nitrogen during the reproductive growth stage. The benefits and applications of solution culture in the study of nodule development and collection of root samples for acetylene reduction assays are discussed.*

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INTRODUCTION

Sand culture has been utilized in numerous studies of nodulation and nitrogen fixation of pasture and grain legumes (Henzell 1962; Wilson 1972; Andrew 1976; Saito *et al.*, 1980; Jones *et al.*, 1981). However, few comparisons appear to have been made concerning the value of solution culture as opposed to other culture methods for such experiments. It is generally assumed that nodulation, vegetative growth, and seed yields are unaffected by various planting media under similar environmental and nutritional conditions, but there is little experimental evidence to support or dispute these assumptions. The present study examines the effects of solution, sand and soil culture on nodulation, growth and seed yield of phasey bean, an annual or biennial tropical pasture legume.

The primary objective of this experiment is to investigate whether phasey bean nodulates freely in nitrogen-free nutrient solution and to obtain preliminary information on the major sites of nitrogen accumulation during the vegetative and reproductive growth stages.

MATERIALS AND METHODS

Experimental Design

The experiment consisted of a factorial combination of three planting media (solution, sand and soil) and six harvests (4, 6, 8, 10, 12 and 14 weeks after planting), arranged in a completely randomized design with four replications. The pots were rerandomized once a month to minimize any positional effects in the glasshouse.

Preparation of Culture Media

Thirty pots (17.5 cm diameter) were each filled with 3.23 kg of a 7 : 1 mixture of fine (0.02 – 0.20 mm) and coarse (0.2 – 2.0 mm diameter) river sand. The sand was previously washed under running water for two days, rinsed with deionized water and then air-dried. Another 30 pots were each filled with 2.63 kg of a yellow podzolic soil (top 15 cm) taken from the University of Queensland farm at Mt. Cotton (Field 7) in southeast Queensland. Available soil nitrogen was determined according to the methods described by Bremner and Keeney (1966), and the level ($NO_3 - N + NH_4 - N$) was 12.4 $\mu g\ g^{-1}$. Soil pH (of 5.0) was measured on a pH meter using 1 : 5 soil to water ratio. The pots containing soil and sand were perforated at the base to allow free drainage. The bulk densities of soil and sand were 1.1 and 1.3 g cm^{-3} respectively.

A third batch of 30 pots was filled with 2.5 litres of nitrogen-free nutrient solution (pH 5.5) of the following composition (μM): K 2743, Ca 1360, Cl 478, S 266, Mg 197, P 114, Na 108, Si 66, Fe (as sequestrene) 12.8, B 9.0, Mn 8.0, Zn 2.2, Cu 1.3, Mo 0.15 and Co 0.02.

Details of the preparation of stock nutrient solutions have previously been described (Wan Mohamad W. Othman 1983). Accurate volumes of each stock nutrient solution were dispensed into the pots, using Jobling dispensers.

Planting and Management

Five phasey bean (*Macroptilium lathyroides* cv. Murray) seeds, scarified and inoculated with *Bradyrhizobium* strain CB 756 (obtained from The Australian Estates Ltd) were sown on 31 July, 1978, in pots of soil and sand, to a depth of 1 to 1.5 cm. Seeds intended for solution culture were placed in a tray of moist sand on the same date, and were transplanted into the previously prepared nutrient solution when the radicles were 2 to 3 cm long. The seedlings were held in 2.5 cm diameter × 2.0 cm deep plant support baskets filled with black polyethylene beads. One seedling was planted in each basket. The baskets fitted snugly into holes in pot covers made of high density black polyethylene. The commercial *Bradyrhizobium* peat culture was added to the nutrient solution one day before transplanting (approximately 6.7×10^5 viable cells ml^{-1}). Initially, five seedlings were planted per pot, these being thinned to one plant per pot two weeks later. Seedlings in the soil and sand cultures were thinned likewise.

All pots were placed on two benches in a naturally lit glasshouse at University of Queensland, Brisbane (Latitude 27° 28′ S). Night temperatures in the glasshouse were maintained between 18 and 22°C by placing four thermostatically controlled electric heaters (each of 2.8 kwatt) close to the benches. The mean maximum temperature throughout the growth period was 27.2°C. The nutrient solution was aerated continuously with compressed air at the rate of about 6 to 7 ml s^{-1}. Deionized water was added to the nutrient solution whenever necessary to ensure that the root systems were completely submerged at all times. The nutrient solution was maintained between pH 5.5 and 5.8 by adding dilute (0.1N) HCl or 0.1 M NaOH once every six or seven days. Stock nutrient solutions were added to the pots of solution culture 6 and 10 weeks after sowing without discarding the original solution. The volumes of stock nutrient solutions added on these two occasions were the same as the initial volumes. The soil and sand cultures were supplied every second day with 150 ml pot^{-1} of identical nitrogen-free nutrient solution. The nutrient solution intended for soil and sand cultures was previously prepared in two 25-litre polyethylene containers, using the appropriate volumes of the stock solutions described earlier. The solid and sand cultures were irrigated to field capacity with deionized water every other day. The diurnal variations in temperatures of soil, sand and solution cultures were recorded once in 8 to 10 days. The mean diurnal temperatures are presented in Table 1.

Plant Height and Non-destructive Nodule and Leaf Counts

Non-destructive nodule counts and other observations on plant growth and development were made every two days on all plants grown in solution culture. Leaf number per plant was also recorded non-destructively every six or seven days. Plant height was measured every two weeks commencing at Week 4. Since the number of pots remaining decreased with time, the above measurements and counts were made on progressively decreasing plant numbers.

Harvesting

Four plants from each culture medium were harvested every two weeks commencing four

TABLE 1
Diurnal variations in temperature (°C) of air (in glasshouse), solution, sand and soil

		Culture media		
Time of day	Air	Solution	Sand	Soil
0800	25.0	18.5	19.0	18.0
1000	27.0	23.0	27.0	26.5
1200	28.0	26.0	30.5	28.5
1400	29.0	29.5	30.0	29.5
1600	26.5	31.0	29.0	28.5
1800	19.0 ←	28.0	24.0	25.0
2000	21.0	26.0	21.0	22.0
2200	20.0	24.0	20.0	20.0

Arrow indicates the start of thermostatically controlled heaters.

The evening and night temperatures were 2 to 4 degrees higher in solution than the soil or sand culture. The reverse occurred at or before noon.

weeks after sowing (Week 4) and ending at Week 14. Ripe pods, if any, were harvested before they shattered. At harvest, the soil and sand were carefully washed off the roots under running water, a sieve being placed underneath to collect any detached nodules or roots. All root systems were thoroughly rinsed in deionized water and blotted dry. The plants were separated into roots, nodules, stems (+ peduncles), leaves, flowers and young pods, and, when present, ripe pods. Ripe pods were further partitioned into hulls and seeds. Tap root length was measured, and the number of nodules, ripe pods, seeds, and leaves per plant were counted. The leaf area was measured using an electronic leaf area meter or Paton Electronic Planimeter (Paton Industries Pty. Ltd., Australia). All plant parts were dried in an oven for 48 hours at 80°C, and later ground in a hammer mill fitted with a 0.25 mm mesh screen.

Plant Analysis (total nitrogen)

Approximately 100 to 200 mg of ground plant material was subjected to Kjeldahl digestion in a 75 ml digestion flask containing 5 ml of concentrated H_2SO_4, one tablet (1 g) of selenium catalyst and 0.5 g sodium thiosulphate. Details of Kjeldahl digestion and the use of an auto-analyzer were described by A.O.A.C. (1965) and Gehrke *et al.* (1973) respectively. Except where otherwise stated, ground plant material from each replication was bulked prior to analysis to reduce the total number of samples required for the assay.

RESULTS

Plant Growth

The length of tap root, plant height, leaf area and number increased with increasing plant age. In all culture media, rapid tap root elongation occurred during early vegetative growth (Week 4 to 8) whereas leaf development and plant height increased rapidly between Week 8 and 12 *(Fig. 1)*, which coincided with the period of active nodulation, except in sand culture in which active nodulation began from Weeks 10 to 14 *(Fig. 3a)*.

In solution and sand cultures, the primary leaves showed mild nitrogen-deficiency symptoms 1 to 2 weeks after planting, but the symptoms disappeared approximately two weeks later. On the majority of plants, the first trifoliate leaf emerged approximately four weeks after planting. Leaf growth (leaf area and number) and plant height were initially better in soil than in sand or solution culture, but with time (beyond Week 10) plants growing in solution culture surpassed those in soil culture in terms of leaf area, leaf number, and plant height (beyond Week 12). However, tap root development was always better in solution than soil or sand culture. During pod-filling and ripening stage (Week 12 to 14) leaf area and number were significantly greater ($P < 0.05$) in solution than soil culture; those in sand culture still lagged behind these two treatments *(Fig. 1)*.

Dry Matter Accumulation

In all culture media, dry matter accumulation in leaves, stems, roots + nodules, and in the whole plant was gradual during the first eight weeks, but increased rapidly between Week 10 and Week 12 for most plant parts with the exception of leaves in sand and soil cultures *(Fig. 2)*. In all treatments, flowering commenced between 9 and 10 weeks after planting. The main feature of the flowering and early fruiting stage (Week 9 to 12) was the sudden increase in the dry matter accumulation in stems and developing fruits. At the end of this stage, the leaves and roots had attained their maximum growth. Although podding began soon after flowering, active vegetative growth continued until Week 12 (with the exception of leaves in sand and soil media), after which it remained constant. Abscission of some lower leaves occurred during the pod-filling and ripening stages. Pods on the basal peduncles ripened rapidly. In solution and soil cultures, ripe pods were the major component of plant dry weight at final harvest. However, in sand culture dry matter accumulated largely in stems and to a smaller extent, in ripe pods. In all culture media, the nodulated roots and stems ceased growth during the pod-filling and ripening stage *(Fig. 2)*.

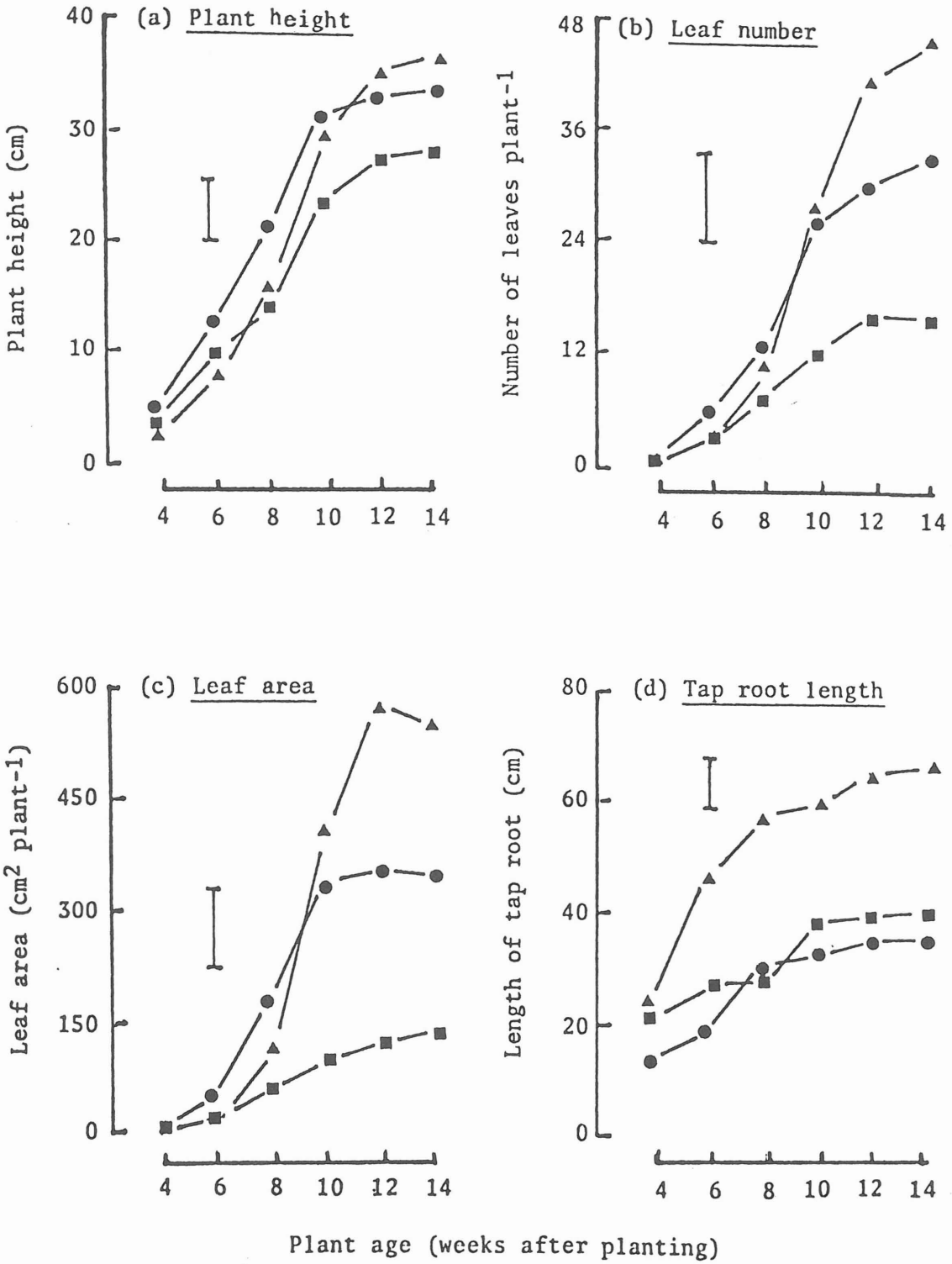

Fig. 1: Effects of solution (▲), soil (●) and sand (■) cultures and plant age on plant height, leaf number, leaf area, and tap root length (Vertical bars denote LSD's at P = 0.05)

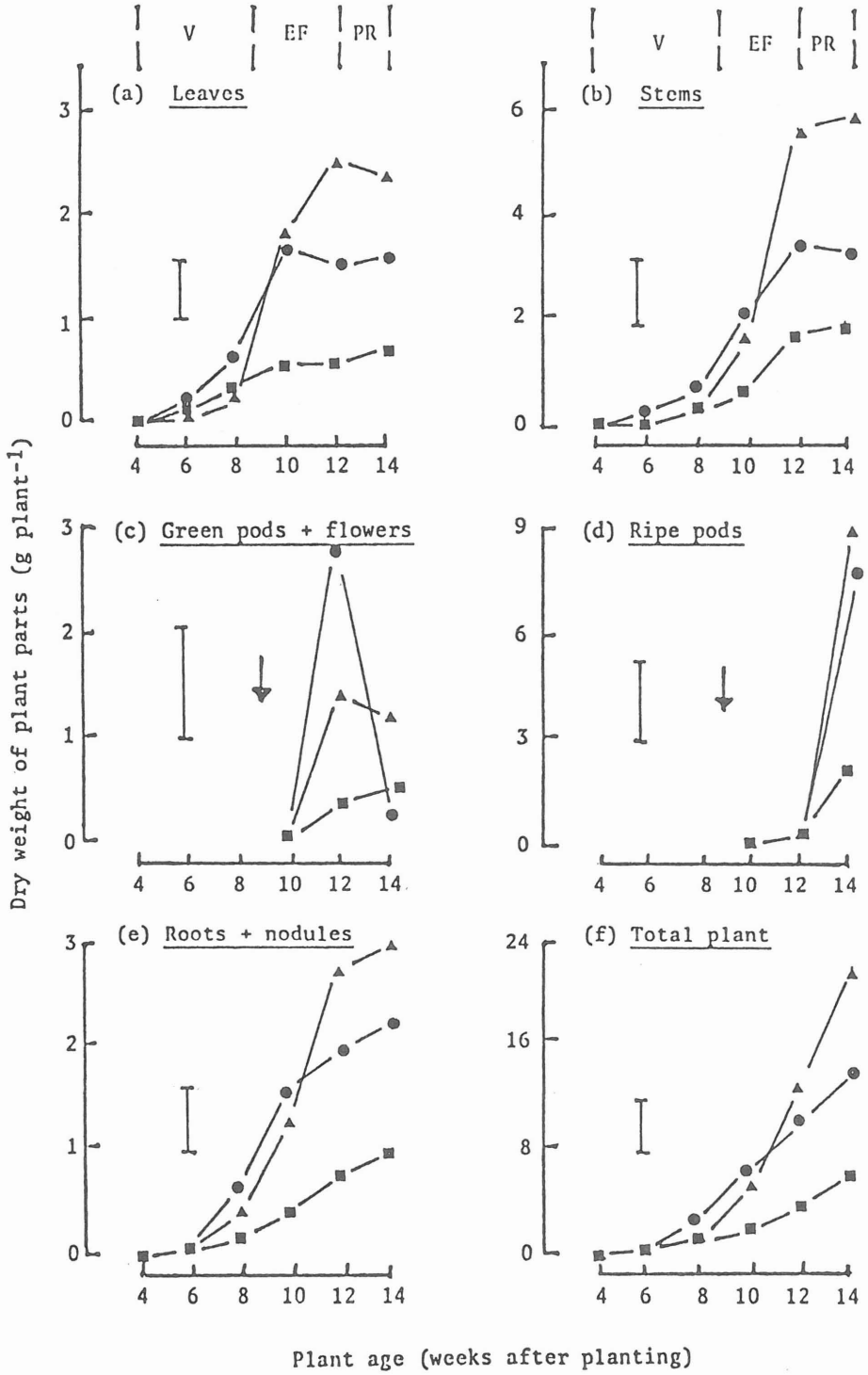

Fig. 2: Dry matter accumulation in vegetative and reproductive parts of phasey bean plants grown in solution (▲), soil (●) or sand (■) cultures (Stages of growth are denoted by: V = vegetative, EF = Flowering and early fruiting, PR = Pod-filling and ripening. Vertical bars denote LSD's at P = 0.01. Arrows indicate commencement of flowering.)

Over the first six weeks there were no significant differences in total dry matter production among the three culture systems. Between Weeks 10 and 14, the total plant dry matter was significantly ($P < 0.05$) higher in soil than in sand culture. Differences between culture media were not significant during vegetative growth. However, beyond the tenth week, the plants in solution culture grew much more rapidly than those in soil culture. Plants in sand culture still lagged behind those in soil culture. At week 14, the roots + nodules, stems, leaves and total dry matter production of solution-grown plants were significantly higher ($P < 0.01$) than those in soil culture *(Fig. 2)*. At this plant age, the dry matter accumulation in the plant parts and in the whole plants was the lowest in sand culture. At final harvest the pods (green and ripe) were the largest single component (46 to 52%) of plant dry weight in all treatments. Of the dry matter gained by the reproductive parts between Week 10 to 14, 80 to 95% accumulated in ripe pods. The seed was the major component (approximately 60%) of ripe pods.

Seed Yields

The mean weight of 1000 seeds (seed size) and total seed yield were significantly higher ($P < 0.05$) in solution than in soil culture (Table 2). However, the other components of seed yield were not affected by either culture. The number of ripe pods and seeds per plant, and seed yield were lower ($P < 0.01$) in sand than in soil culture, but the mean weight of 1000 seeds was significantly greater ($P < 0.05$) in sand culture. Seed number per pod was not significantly affected by the culture medium.

Nodulation

In solution culture, the first nodule was visible within 16 days of sowing. The first nodule often formed on the tap root 5 to 6 cm below the plant support baskets. Newly formed nodules were initially small and white in colour and turned pink within six to seven days. As the plant developed, the pink nodules increased in number and size. In all treatments there was a sudden increase in nodule number between Week 8 and 10 (prior to and during flowering), but nodule development was apparently retarded during the pod-filling stage as in the solution and soil culture media *(Fig. 3)*. Although nodule number was generally similar in all culture media, the mean weight per nodule (nodule size) and total nodule weight were significantly higher in solution culture than sand or soil culture. Total nodule dry weight and nodule size were significantly suppressed by sand culture, especially between Week 12 and 14.

In solution culture the roots were observed to be suberized, and the surface of nodules developed extensive lenticels (spongy tissues with large intercellular spaces). Nodules in soil and sand cultures developed relatively few lenticels.

Nitrogen Fixation and Accumulation in Plant Parts

In all culture media, nitrogen accumulation in vegetative parts increased in a sigmoid

TABLE 2

Effects of solution, soil and sand cultures on components of seed yield (14 weeks after planting)

Culture media	No. of ripe pods plant^{-1}	No. of seeds pod^{-1}	No. of seeds plant^{-1}	Mean wt. of 1000 seeds (g)	Total seed yield (g plant^{-1})
Solution	31.8	19.8	630	8.71	5.49
Soil	32.0	18.6	595	7.66	4.56
Sand	7.7	19.4	150	9.00	1.35
LSD ($P = 0.05$)	7.3	0.81	129	0.65	0.92

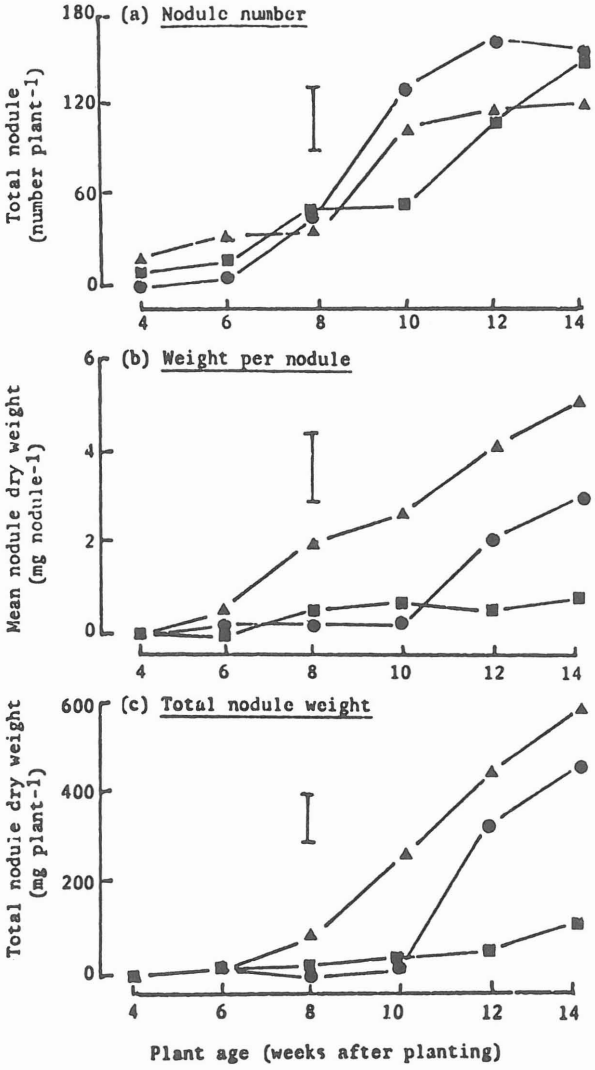

Fig. 3: Effects of solution (▲), soil (●) and sand (■) cultures and plant age on nodule number, nodule size and total nodule dry weight (Vertical bars denote LSD's at P = 0.05.)

manner, but total plant nitrogen increased exponentially from Week 4 to 14 *(Fig. 4)*. Very rapid increases in total plant nitrogen occurred during the flowering and early fruiting stages. Prior to or during these stages the leaves and stems were the major sites of nitrogen accumulation. However, the pods gained substantial amounts of nitrogen during the reproductive stage. At Week 14 the pods (green + ripe) of plants growing in solution, soil, and sand culture contained 52%, 55% and 51% of the total plant nitrogen, respectively (equivalent to 372, 276 and 106 mg N plant $^{-1}$). Of the nitrogen gained by the reproductive parts at final harvest, 63 to 69% ended up in seeds *(Fig. 4d)*. During the reproductive growth stage, nodulated roots (roots + nodules) gained approximately 10 to 20% of total plant nitrogen.

Since nitrogen from seed source was very small (approximately 0.5 mg N seed $^{-1}$), and there was no deliberate application of nitrogen fertilizer (other than N naturally occurring in soil), total nitrogen in the whole plant *(Fig. 4f)* can be regarded as a close approximation to symbiotic nitrogen fixation. Nitrogen fixation was apparently unaffected by the various culture media during the vegetative growth. However, the rate of nitrogen fixation was significantly higher in solution than in sand culture, especially during the reproductive stage *(Fig. 4f)*. Although the rate of nitrogen fixation was consistently higher in solution than in soil culture during the reproductive growth stage, differences were significant only at Week 14.

DISCUSSION

Effects of Nodulation and Nitrogen Fixation on Dry Matter Production and Seed Yields

Plant growth was initially slow in solution and sand cultures probably due to nitrogen starvation before the establishment of effective symbiosis. The growth pattern of component parts of phasey bean plants and the strong sink effect of pods for nitrogen and carbon (dry matter) were similar to those reported for cowpea and soybean (Herridge 1977; Warembourg *et al.*, 1982). However, in phasey bean the growth of leaves, stems and roots declined very little during the pod-filling and ripening stage. This lack of decline in vegetative growth was associated with the continual increases in nodule dry weight and in the rates of nitrogen fixation during the period of rapid seed development *(Figs. 2, 3c, 4f)*. Improved plant growth in solution culture, relative to the growth in other media, was apparently associated with increased nodulation and higher rates of nitrogen fixation *(Figs. 2, 3, 4f)*. Results of the present experiment

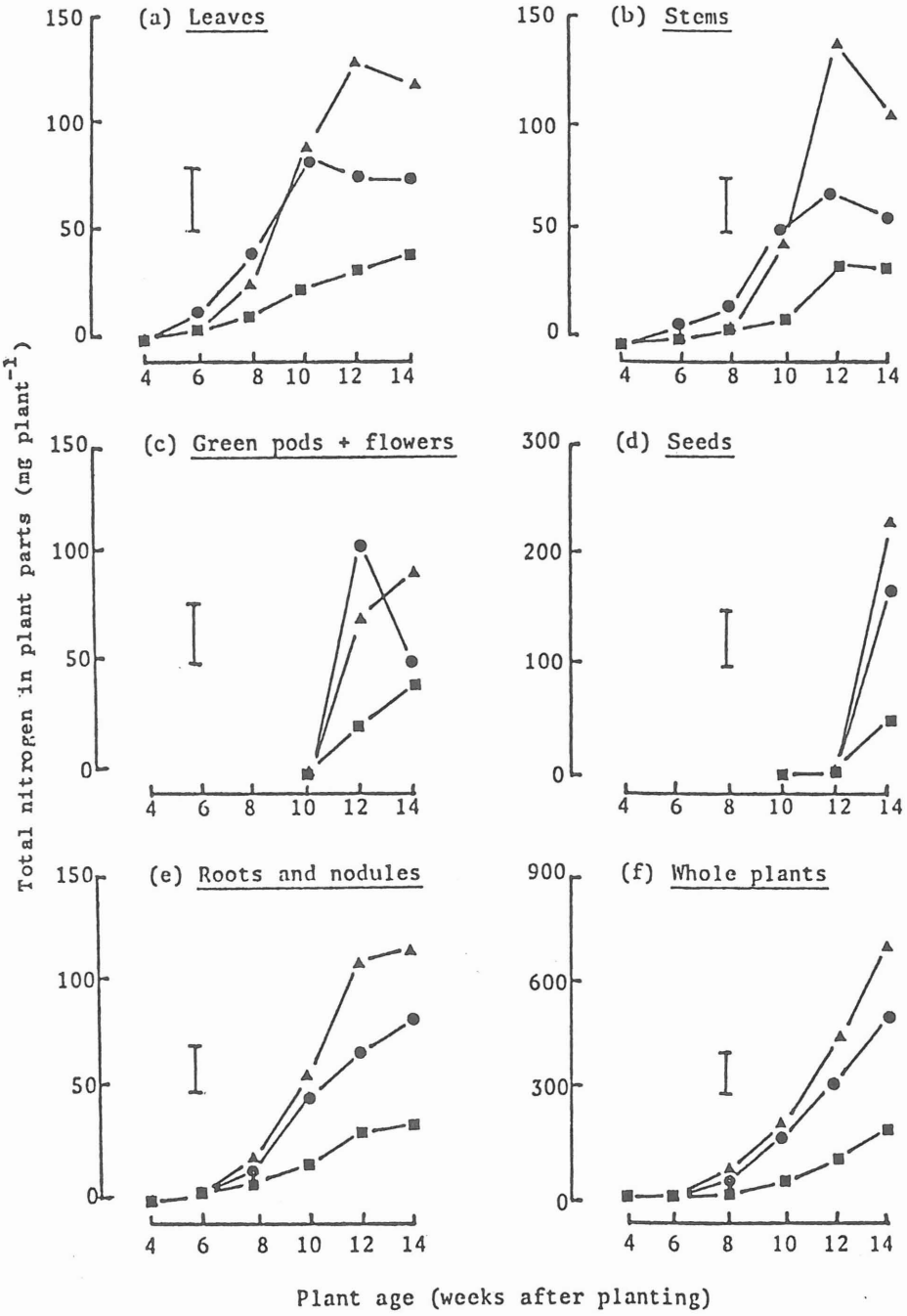

Fig. 4: Nitrogen accumulation in leaves, stems, pods, seeds, root and nodules, and whole plants in phasey bean plants grown in solution (▲), soil (●) or sand (■) cultures. (Vertical bars denote LSD's at P = 0.05.)

support those of Franco and Munns (1982) who showed that nitrogen fixation (acetylene reduction assay) and growth of French bean were better in solution culture than in gravel culture.

In the present study, the mean day/night temperatures (20/27.2°C) in the glasshouse were 3 to 5 degrees below the optimum requirement for vegetative growth of phasey bean (Whiteman 1968). Differences in air temperature have been known to cause marked changes in nodule weight and nodule size of cowpea (Dart and Mercer 1965). It is highly unlikely that air temperature was responsible for the differences in nodulation and growth of phasey bean reported in the present study, since all plants were subjected to the same ambient temperature. The reasons for increased root growth and nodulation in solution culture are not well understood.

The slow nodule development during the initial stages of plant growth *(Fig. 3)* is consistent with the findings of other studies on other legumes, both in the field and under controlled environmental conditions (Diatloff and Ferguson 1970; Nicholas and Haydock 1971; Wilson 1972). Nodule development of Glycine was also found to be retarded in siliceous sand, and in yellow podzolic soil, taken from a similar site, compared to that in black earth (Thomas and Whiteman 1971).

The depressed nodulation in soil culture during the first few weeks of planting *(Fig. 3)* was probably due to the inhibitory effects of soil mineral nitrogen. Recent investigations indicated that high levels of soil mineral nitrogen suppressed nitrogen fixation of other legumes (Alston and Graham 1982). There is evidence to suggest that mineralization of soil nitrogen depresses nodulation of some legumes (Diatloff 1967; Quilt and Dalal 1979) or replaces symbiotic nitrogen fixation of other legumes (Allos and Bartholomew 1959).

The distinct development of lenticels on the surface of nodules of solution-grown plants in the present experiment, and the growth of massive lateral roots of phasey bean under flooded conditions in other studies (Siregar 1977; Chudasama 1981) are features associated with the physiological adaptation of this plant to the wet culture system. Lenticels are often observed on nodules of other legumes growing in water logged conditions, and they are reportedly associated with the gaseous exchange in and out of nodules in a wet environment (Pankhurst and Sprent 1975; Minchin and Summerfield 1976).

The data indicate that differences in root environment (e.g. temperature, aeration, and the physical nature of the rooting media) contribute to marked variations in nitrogen fixation which in turn affect vegetative growth and seed yields *(Figs. 2f, 4f,* Table 2). The 1000 seed weight, and seed number per pod obtained in the present study are comparable with those reported by Humphreys (1979). The marked decline in total nitrogen in stems of solution-grown plants between Week 12 and 14, despite large increases in nitrogen fixation *(Fig. 4b, d, f),* was probably associated with the remobilization of nitrogen to the developing seeds which were produced in relatively large quantities in solution culture. With phasey bean there seemed to be little loss (remobilization) of nitrogen from the leaves during pod-filling stage *(Fig. 4a).* It appears that high rates of nitrogen fixation and the partitioning of nitrogen into various plant parts are major factors influencing vegetative growth and reproductive development.

Benefits of Solution Culture Techniques

Results of the present study indicate that nitrogen-free solution culture enhances potential nodule development, particularly nodule size *(Fig. 3b)* which makes nodule counting much easier. Harvesting and root-washing of solution-grown plants were completed within 1 to 2 minutes per sample compared with 25 to 30 minutes with the other media. One person was able to wash 35 to 40 excised root systems within one hour of harvest. With reference to acetylene reduction assays, root incubation with acetylene could be done quickly without having to stagger the harvesting or incubation, thereby eliminating differences due to diurnal fluctuations in nitrogen fixation or nitrogenase activity.

Another major advantage of solution culture is that nodulation and nodule development can be observed and recorded non-destructively. Also, at harvest, all roots and nodules of solution-grown plants can be recovered easily. These findings illustrate the suitability and importance of solution culture as one of the experimental techniques in future experimental work, concerned with the accurate study of nodulation and nitrogen fixation of legume plants during ontogeny or following defoliation.

CONCLUSION

Nitrogen-free solution culture enhanced nodulation largely by increasing nodule size. The rate of nitrogen accumulation in solution-grown plants was greater than that in plants grown in soil or sand culture. Sand culture suppressed nodulation, plant growth and seed yield.

During early plant growth, nitrogen accumulated largely in the leaves and stems, but pods were major sites of nitrogen accumulation during pod-filling stage. Roots and nodules of solution-grown plants could be harvested easily and rapidly without loss.

ACKNOWLEDGEMENTS

The authors would like to thank the Australian Development Assistance Bureau for providing the necessary funds for this study. The financial support was arranged through the technical co-operation of the Australian Universities International Development Programme in collaboration with Universiti Pertanian Malaysia, the home university of the senior author.

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(Received 13 January, 1986)