Harnessing Biological Nitrogen Fixation in African Agriculture

Challenges and Opportunities

edited by
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and
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PART 5: BIOLOGICAL NITROGEN FIXATION IN GRAIN LEGUMES

Part 5
Biological Nitrogen Fixation in Grain Legumes

5.1 Nodulation and yield of some self-nodulating soyabean varieties in Malawi

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Summary

Natural nodulation and yield of self-nodulating soyabean (*Glycine max*) varieties were evaluated in trials on different soils of Malawi. Treatments were: (a) seed inoculation with *Bradyrhizobium japonicum*, (b) 200 kg/ha S-mixture of NPK (6-18-6), (c) inoculation plus 200 kg/ha S-mixture and (d) no inoculation. Data obtained indicate that nodulation of two self-nodulating cultivars Magoye and Hernon 147 was ubiquitous but intensity varied with soil types and improved as the season progressed. In some areas nodulation was very profuse with more than 40 nodules per plant while other sites registered a single nodule per plant or none at all. Observation plots including promiscuous lines from IITA also indicated varied response across sites, depending upon soil type. Seed inoculation improved nodulation of all the varieties. Inoculation plus fertilising with phosphate at 200 kg/ha S-mixture gave the best response in terms of nodule numbers, dry weight of nodules and seed yield. To achieve high grain yields in excess of 1 500 kg/ha, it is recommended that these self-nodulating soyabean varieties be inoculated to boost nodulation and plant vigour early in the growth cycle, especially on virgin land.

Introduction

The cultivation of soyabeans in Malawi requires that seed should be inoculated with a highly effective strain of the nodule-forming bacteria *Bradyrhizobium* spp. This type of bacteria is not known to occur naturally in the soils of Malawi. However, some varieties developed in Zambia, namely Magoye and Hernon 147 are reported to form nodules, fix nitrogen and give good grain yields without being artificially inoculated (Javaheri, 1986; Javaheri and Joshi, 1986).

In Malawi we do not have such varieties released to farmers. This is mainly a result of lack of scientific information on the performance of these varieties on the various soil types of Malawi. Other workers, e.g. Peoples and Craswell (1992) present a view that although such soyabeans are able to nodulate with many strains, they may be able to fix nitrogen only with a limited number of them.

Furthermore, some soils may be devoid of rhizobia capable of an effective symbiosis. In addition, differences in soil environments are likely to be very influential on the host-strain interaction. As such it is important and appropriate that these varieties be evaluated locally prior to mass release to farmers.

The objective of this experiment was to assess the nodulation and yielding ability of the self-nodulating varieties Magoye and Hernon 147 in soils of Malawi without artificial inoculation with rhizobia.

Materials and methods

Experimental design

The experimental design was a 3 x 4 x 4 randomised block design. Self-nodulating soyabean varieties Magoye, Hernon 147 (H147) and a local variety, Geduld, which requires inoculation, were used. Inoculation treatments were as follows: 1. seed inoculation with MG 614 (USDA 110) plus 200 kg/ha " S mixture", NPK (6-18-6) basal fertiliser dressing; 2. no seed inoculation; but plus 200 kg/ha "S mixture" only; 3. no seed inoculation; no " S mixture "; 4. inoculation with MG 614 only. Each gross experimental plot measured six metres long and consisted of four ridges spaced 90 cm apart. The net plot consisted of two middle ridges with one metre discarded off the end of each ridge, leaving only four metres for grain yield determination at the harvest. Seeds were sown at the recommended spacing of two rows per ridge with rows 15 cm apart and seeds 5 cm apart within the row; giving a seed spacing of 40 seeds per metre of row.

Determination of soyabean nodulation

Plant samples for nodulation were collected at either 2 weeks and 4-6 weeks or at 8 weeks after planting. Sampling was done by carefully digging out five randomly selected plants from either of the two outside ridges of each plot. Care was exercised to retrieve as much of the root system with nodules intact as possible. Soil was carefully shaken off and samples were placed in paper bags and taken to the laboratory. Nodules were then plucked off by hand, counted and then weighed to obtain fresh weight. Size of nodules was determined by placing each of five randomly picked nodules on a graph paper with a millimetre grid and then noting the number of millimetres occupied by each nodule. Nodules were then dried in an oven set at 70°C for 24 hours. Thereafter nodule samples were weighed to determine nodule dry matter.

Determination of plant shoot dry matter and nitrogen content

All the above-ground plant samples were dried for 48 hours in an oven set at 70°C and weighed to determine dry matter yield. For nitrogen determination, sub-samples were ground, placed in plastic bottles and subjected to chemical analysis using the Kjeldhal method. However, due to a fire which damaged the Chemistry Laboratory, data of plant chemical analyses will not be available since analyses were not completed.

Determination of Rhizobium numbers in the soil

Estimated numbers of initial populations of *Bradyrhizobium japonicum* in soils at experimental sites were determined from soil samples collected from 0-15 cm and 30 cm depths of each plot using the plant infectivity Most Probable Number (MPN) technique of Vincent (1970). All soils including those at observation sites showed less than 1 000 rhizobia cells/g of soil, the critical minimum level for successful nodulation of soyabeans.

Experimental sites

Experimental sites were Chitedze Agricultural Research Station (Block NR2), a farmer's field near Lisasadzi in Kasungu Agricultural Development Division (ADD), Kaluluma (Kasungu ADD) and Zombwe (Mzuzu ADD). Soil chemical data for the sites are presented in Table 5.1.1.

Harvesting and grain yield determination

At physiological maturity, plants from the net plot were removed by hand, placed in sacks and allowed to sun-dry before threshing and weighing to determine yield. Five sub-samples were taken to determine moisture content using a Digital Grainmaster Moisture Meter. All grain yield data were adjusted to 8% moisture content which is recommended for reporting soyabean yield.

Observation plots

In 1990/91 some 25 sites across Malawi were planted to ascertain the nodulation response of Magoye and Hernon 147. These plots were established by the Christian Service Committee as part of their campaign to popularise soyabeans as a crop for human consumption to combat protein malnutrition in rural Malawi.

Table 5.1.1: Initial soil chemical data for the experimental sites

Parameter	Experimental Sites			
	Chitedze	Kaluluma	Lisasadzi	Zombwe
$pH_{(water)}$	4,9	6,2	6,5	5,6
Textural class	sandy loam	sandy loam	sandy loam	sandy loam
Organic matter %	4,34	0,75	0,84	1,4
% Carbon	2,52	0,44	0,49	0,81
% Nitrogen	0,22	0,04	0,04	0,07
C/N ratio	11,6	12,0	12,5	11,4
P (Bray 1) *µg/g*	3,81	39,3	22,0	0,78
Exchangeable cations (meq/100g):				
Ca	5,36	1,20	1,50	0,80
Mg	1,05	0,40	0,70	0,64
K	0,34	0,38	0,25	0,48
Na	0,08	0,04	0,04	0,03
H	nd	0,77	0,75	nd
TEB *(meq/100g)*	6,99*	2,01	2,49	1,95*
CEC *(meq/100g)*	nd	2,78	3,24	nd
% Base saturation	nd	72,0	77,0	nd

nd = not determined
* Without Al + H

Glasshouse pot observation of IITA promiscuous lines

In 1993/94 Magoye, Hernon 147 and Geduld together with 11 lines from IITA were observed in a pot study for natural nodulation in two different soils under aseptic conditions: soil from Chitedze and soil from Bembeke. Nodulation was assessed at six weeks after planting.

Results and discussion

Rainfall

During 1990/91 there was adequate rainfall for soyabean cultivation in Malawi. 1991/92 Malawi experienced a severe drought although some early planted crops managed to give reasonable yields in excess of 1 000 kg/ha. The following season 1992/93 had good rainfall adequate for soyabean. 1993/94 season was another drought season with rains far below the expected amounts such that soyabeans were severely affected at most sites in Malawi.

Nodule numbers

In all the seasons at all sites, there were significant differences in the response of the varieties in terms of the number of nodules formed (e.g. Fig 5.1.1.). At Chitedze, variety Geduld formed more nodules than either Magoye or Hernon 147 in all treatments except the uninoculated control (data not shown). At this site it was important to treat all the varieties with some inoculation or with phosphate fertiliser.

In 1993/94 at all sites except Kaluluma, it was beneficial to have Magoye inoculated. In the previous seasons, inoculation increased nodulation across all the sites except Lisasadzi where the differences were more pronounced when inoculation with MG 614 was reinforced with the application of 200 kg/ha S -mixture as a phosphate basal dressing. At Lisasadzi, application of the phosphate fertiliser alone suprisingly reduced nodulation.

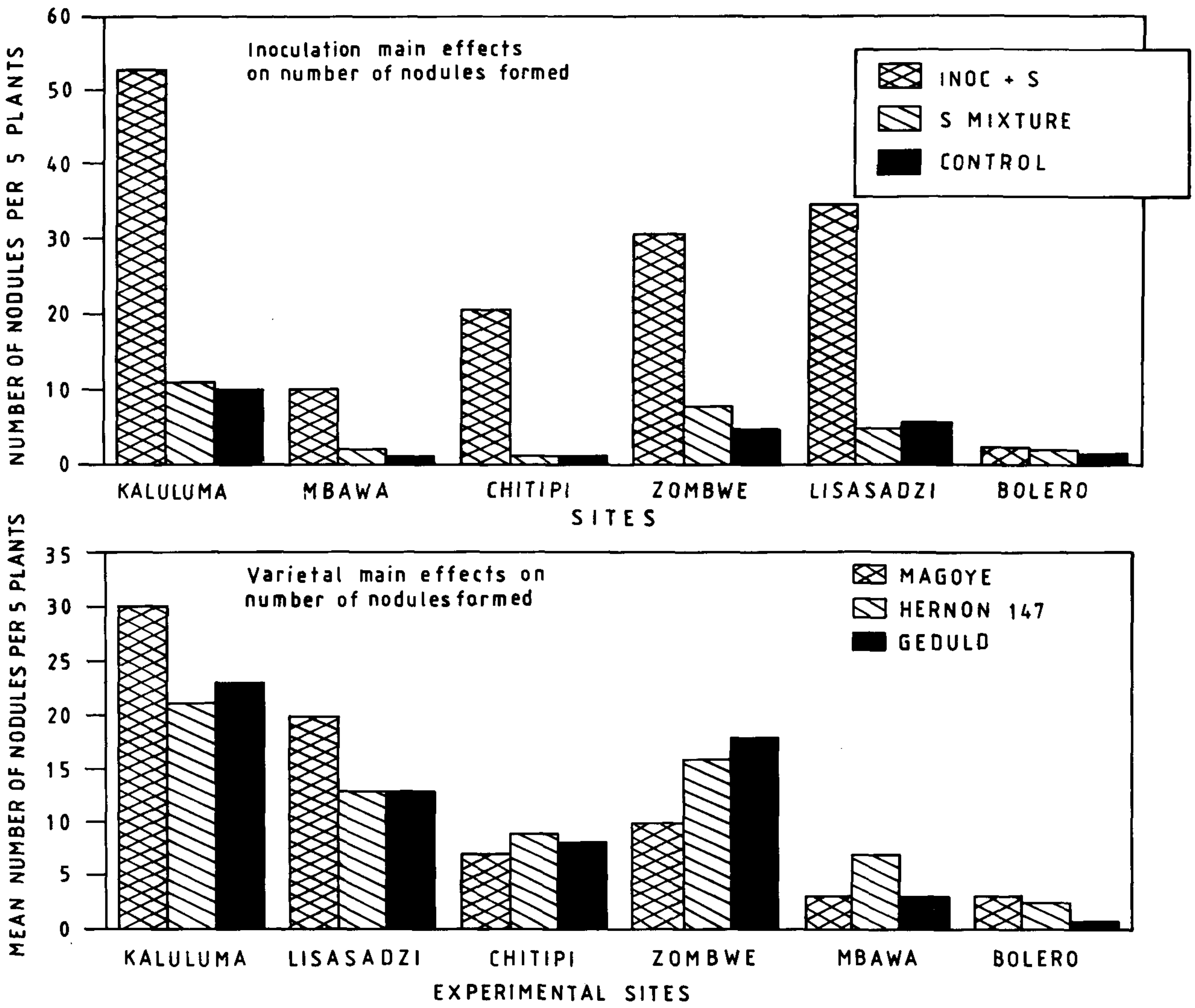

Fig. 5.1.1: Nodulation response of Magoye, Hernon 147 and Geduld (local check) as influenced by the main effects of inoculation (above) or variety (below) at selected sites, 1991/92 season

The data across the sites, show that for good formation of nodules regardless of the variety, it is worthwhile to inoculate with MG 614 and add some phosphate fertiliser in the form of S-mixture.

Again in 1993/94, comparing the four sites, the varieties formed more nodules at Lisasadzi and Kaluluma than at either Chitedze or Zombwe. As in the preceeding season, the least number of nodules was formed at Zombwe which was a virgin site and had lower rainfall and less soil phosphorus than the other sites.

The formation of nodules in the uninoculated treatments with Geduld which is specific in its rhizobial requirements, was probably due to cross-contamination during field operations. This was more evident at Lisasadzi under farmer management where there was close supervision of field operations, especially weeding. What is evident from the nodulation data is that the population of *Bradyrhizobium* spp. and *Rhizobium* spp. in soils of Malawi are not uniform. As such, nodulation of promiscuous soyabeans will also depend on the initial levels of indigenous populations of these nodule-forming bacteria.

Size of nodules and nodule dry weight

In the previous season, Magoye had a greater nodule mass than either Hernon 147 or Geduld at all sites except Chitedze. However, in 1993/94 season, this variety yielded less nodule mass than the other varieties, especially when either inoculated or fertilised. The uninoculated treatment tended to give smaller nodules than the treated plots (Fig. 5.1.2).

Root dry weight

At all the experimental sites, significant differences in root dry weight were obtained between the varieties ($p < 0.05$) (data not shown). However, like in the preceding season, Chitedze had bigger root mass than at the other sites, probably due to better land preparation with tractor ploughing unlike hoe tillage at the other sites.

Generally, application of 200 kg/ha 'S' mixture improved root mass formation at all the sites. This effect on root mass would be expected since the fertiliser was supplying mostly phosphorus which is an essential nutrient for root development. However, treatment effects were not clearly discerned probably due to the drought.

Dry matter of plant shoots

There was no significant influence of either the inoculation treatment or the type of variety on dry matter of plant shoots at Chitedze and Lisasadzi (data not shown). At Zombwe, Magoye had significantly lower dry matter yield when either not inoculated or not fertilised. A similar trend was observed at Kaluluma.

Pod yield

The highest pod yields in 1993/4 were obtained when the seeds were fertilised. Inoculation had no significant effect on pod yield at the three sites. In previous seasons, a similar response was obtained, suggesting that inoculation treatments and biological nitrogen fixation *per se* were not the major factors influencing pod formation and pod fill.

Grain yield

Typical grain yield data are given in Fig. 5.1.3. In 1993/94 the trial at Lisasadzi was harvested by the farmer and seed grain was bulked. As such no grain yield data are available from this site. At all sites Magoye responded to both inoculation and fertilisation. Geduld responded similarly unlike in the preceding season when it was superior in all the treatments and clearly outyielded the two self-nodulating varieties Magoye and H147. In the preceding seasons no major differences could be noticed between the two self-nodulating varieties.

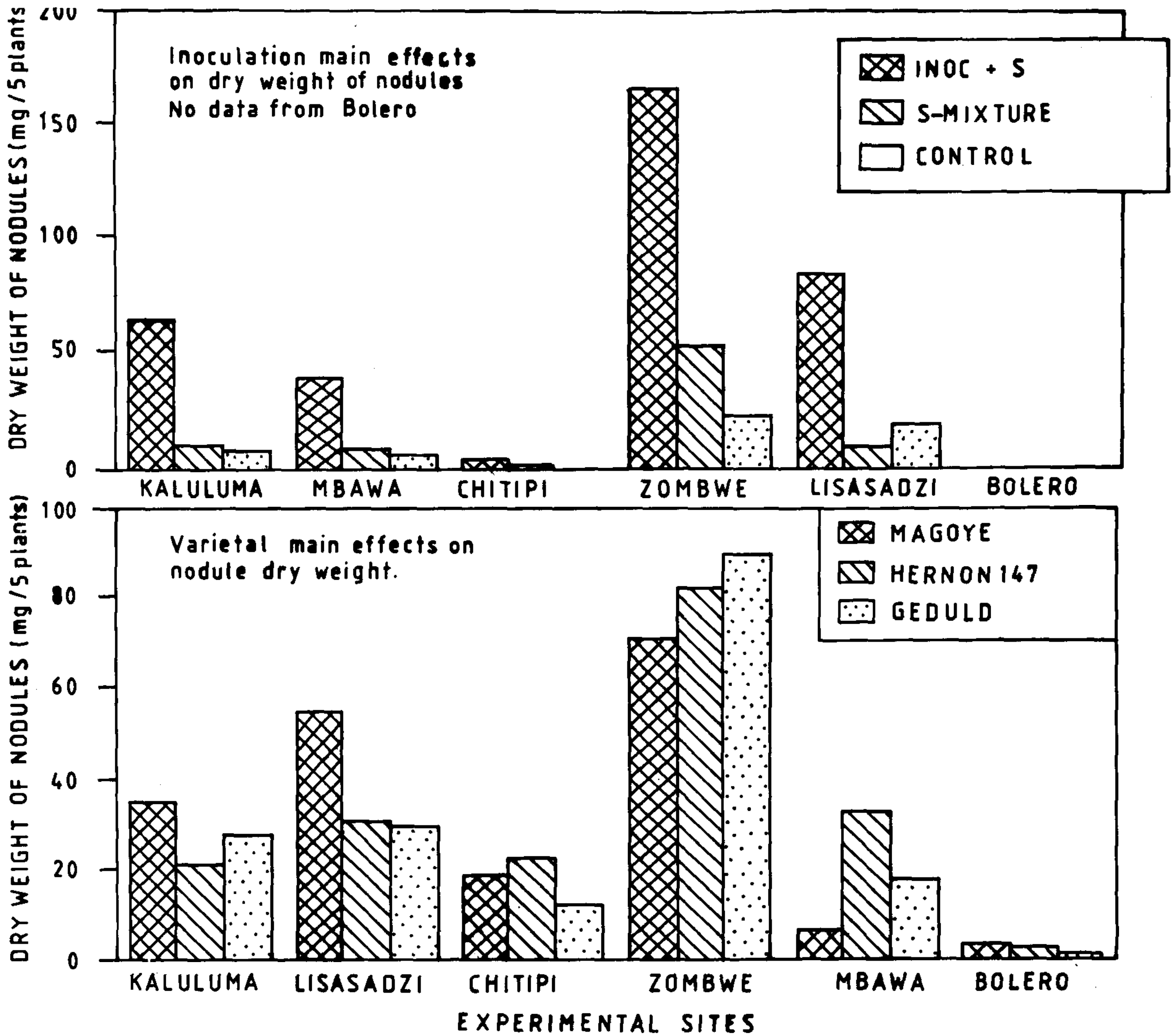

Fig. 5.1.2: Nodulation response in terms of dry weight of nodules of Magoye, Hernon 147 and Geduld (local check) as influenced by inoculation effects (above) and variety (below) at selected sites, 1991/92 season

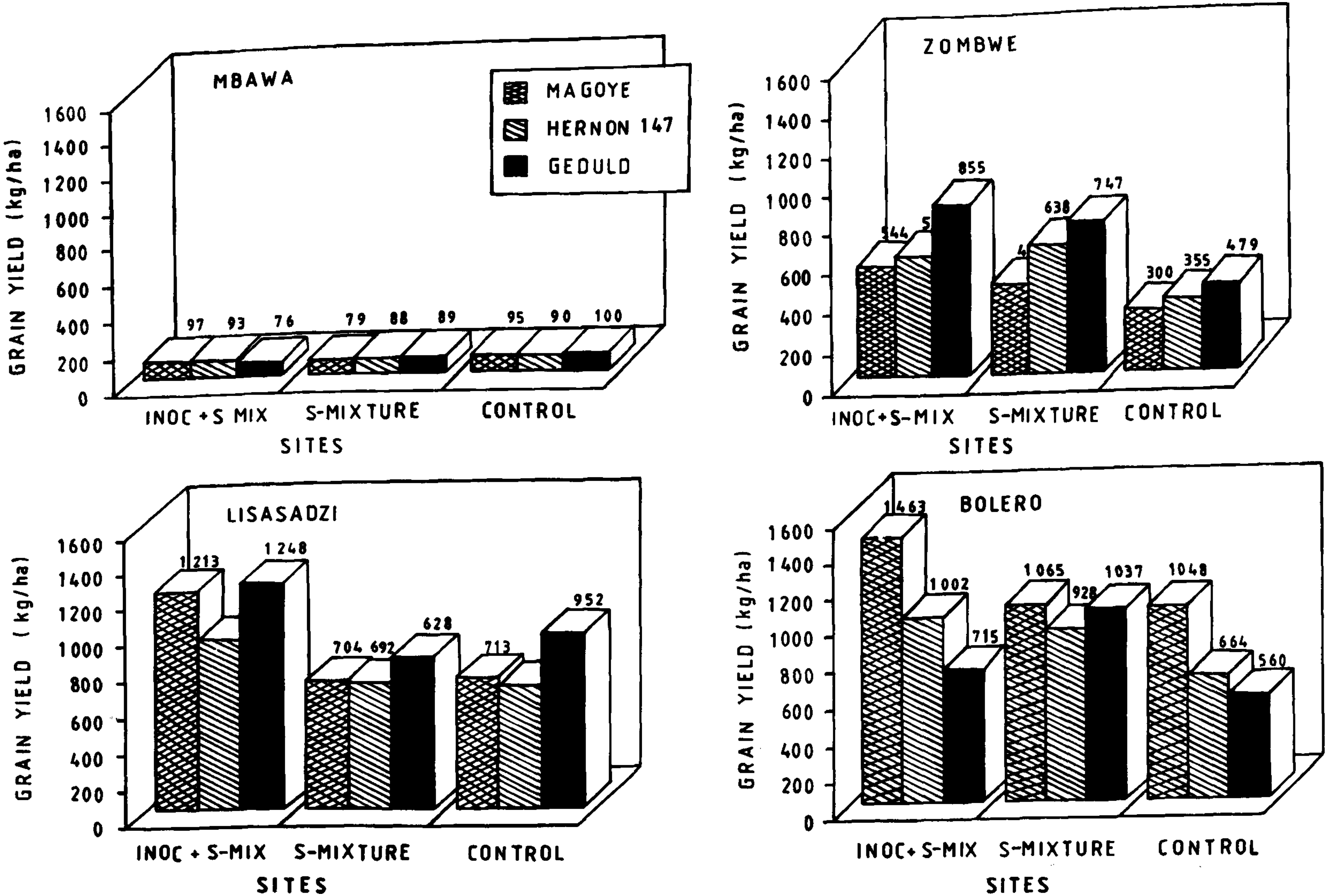

Fig. 5.1.3: Grain yield responses of Magoye, Hernon 147 and Geduld (local check) at four selected sites as influenced by inoculation and fertilisation, 1991/92 season. The control plots received no fertilizer and no rhizobium inoculant

Above all, Chitedze had better yields than the other sites. This could be attributed to earlier planting at Chitedze and different amounts of rain across the sites.

Nodulation and grain yield in observation plots

Data in Tables 5.1.2 and 5.1.3 give nodulation response across several sites in observation plots and in a glasshouse pot study respectively. It will be noted in Table 5.1.2 that nodulation ranged from no nodules to 268 per plant. In the pot study the nodulation of the "self-nodulating" lines also varied depending on the sites, with more nodules at Bembeke than at Chitedze. In the pot study Magoye, TGX 1456-2E, TGX 1445-2E, and TGX 1448-2E had good nodulation at both sites. However, the nodulation of Geduld at Bembeke is questionable. It is likely that Bembeke soil was once a site for previous inoculation studies since this site is an old field at an experimental station. Initial MPN counts showed less than 1 000 *Bradyrhizobium japonicum* cells/g of soil at this site.

Table 2: Nodulation of self-nodulating lines in observation plots across Malawi, 1990/91 season

Site	No of Nodules/plant
1. Ngononda (Dedza)	44
2. Bembeke (Dedza)	27
3. Ndanje (Zomba)	33
4. Kame (Ntcheu)	53
5. Kapalamula (Machinga)	51
6. Sawali (Machinga)	37
7. Nchatu (Mulanje)	88
8. Chigwembere (Mulanje)	268
9. Mtunthama (Mulanje)	204
10. Ekwaiweni (Mzimba)	13
11. Mifumo Church, Ndanje (Zomba)	112
12. St Kizito Church (Machinga)	158
13. Emsizini (Mzimba)	14
14. Ekwendeni (Mzimba)	0
15. Ekwendeni, Mr Ngulube's farm (Mzimba)	87
16. Mdere, (Machinga)	43
17. Puteya (Zomba)	185
Mean across sites	83,4

Data from 10 m x 10 m observation plots on farmers' gardens in 1990/91 season.
Source: S. Carr, Christian Service Committee of the Churches in Malawi, Private Bag 5, Zomba, Malawi.

Conclusions

The major objective of this trial was to evaluate the growth and yielding capability of the new varieties Magoye and Hernon 147 when grown without artificial inoculation. Some new promiscuous lines from IITA were also evaluated. This was aimed at ascertaining whether Magoye and Hernon 147 would be well adapted to the various ecological areas of Malawi. Such information is useful in deciding the release of any new crop variety to the farmers.

The data obtained have shown that the varieties do nodulate freely with the indigenous rhizobia in the soils at the sites used and at several observation sites across Malawi. However, nodulation is not as profuse as when inoculated. In some of the preceding seasons, without any inoculation, grain yield was less than 1 000 kg/ha for all the three varieties. In such seasons, however, under no inoculation the local control Geduld which requires artificial inoculation was the highest yielder except at one site. The inoculation effects were somehow confounded with the basal application of 200 kg/ha S-mixture. In the 1993/94 season, drought had a devastating effect on most parameters studied, especially grain yield.

Table 5.1.3. Nodulation in potted soils of IITA self-nodulating lines

Variety	Chitedze Soil	Number of nodules per plan Bembeke Soil	Variety Means
1. GEDULD	0,0	8,8	4,40
2. HARDEE	0,0	1,3	0,65
3. HERNON 147	0,8	9,0	0,85
4. MAGOYE	1,5	27,3	28,05
5. TGX 1519-1D	0,5	4,3	2,40
6. TGX 1445-2E	0,9	13,0	6,95
7. TGX 1447-3D	0,0	1,3	0,65
8. TGX 1478-2E	0,0	2,3	1,15
9. TGX 1445-3E	1,0	7,3	4,15
10. TGX 1456-2E	0,7	22,0	11,35
11. TGX 1448-2E	0,3	8,5	4,40
12. TGX 1463-1E	0,9	3,5	2,20
13. TGX 1437-1D	0,0	0,8	0,40
14. TGX 1485-1D	0,0	0,8	0,40
15. TGX 1470-1D	2,0	2,0	2,00
Site means		0,57	9,42

From the above information it can be concluded that these new varieties have some potential. However, for assurance to achieving high yields, fertilisation and/or inoculation are essential. This conclusion is consistent with research done in Zambia (Munyinda *et al*, 1988; Javaheri, personal communication). Their data show that Magoye responds well to inoculation and was superior to varieties Bossier and Santa Rosa when inoculated with rhizobium.

Considering the low cost of inoculating soyabeans at MK11,00/ha = US$1,29/ha), it is advisable to inoculate all soyabean varieties in Malawi so as to achieve high grain yields in excess of 1 500 kg/ha. The superiority of the self-nodulating varieties Magoye and Hernon 147 over Geduld in terms of grain yield under no artificial inoculation has not been demonstrated across sites, despite good nodulation at mosts sites in Malawi.

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