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The improvement of crop yield in marginal environments using 'on-farm' seed priming: nodulation, nitrogen fixation, and disease resistance

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Abstract. On-farm seed priming with water is a low-cost, low-risk technology that is easily adopted by resource-poor farmers. It increases the yield of tropical and subtropical annual crops in marginal areas by a combination of better crop establishment and improved individual plant performance. The effects of seed priming, i.e. soaking seeds overnight in water before sowing, on plant growth and development are consequences of faster germination, emergence, and more vigorous early growth. Results from *in-vitro*, on-station and on-farm experiments are discussed.

Recent work has tested opportunities for resource-poor farmers to use seed priming as a vehicle for applying biofertilisers (Rhizobia). Preliminary results from field experiments suggest that these interventions are very effective over and above the already demonstrated benefits of priming with water alone. In a pot experiment using chickpea, combining a *Rhizobium* inoculation with seed priming significantly increased nodulation but had little effect on yield. Nevertheless, the results confirmed that *Rhizobium* inoculation is compatible with on-farm seed priming.

Observations in the field have shown that some primed crops show enhanced resistance to disease, either as a consequence of increased vigour, altered phenology, or due to some more fundamental mechanism associated with exposure of seeds to anaerobic conditions during priming. Priming seeds of a highly susceptible cultivar of pearl millet in water for 8 h before sowing significantly reduced the incidence of downy mildew in artificially infected seedlings from 80% to less than 60%.

Additional keywords: pearl millet, downy mildew, chickpea.

Introduction

Good crop-stand establishment is vital in the production of annual crops from seed because patchy stands result in low yields. Having good stands is particularly important for resource-poor farmers in developing countries because, even if sparse crops can be re-sown, it is expensive and can lead poor farmers into crippling debt. Good crop establishment is especially difficult in marginal, rainfed environments where many poor farmers live. Several factors, e.g. unpredictable and erratic rainfall, poor soils, low-quality seed, and limited availability of labour or draft power contribute to a situation in which good crop establishment is often the exception rather than the rule (Harris 1992, 1996).

Since 1990, we have looked in detail at the performance of 'on-farm' seed priming—soaking seeds in water before sowing—in a range of tropical and subtropical crops (Table 1). This simple technology, as expected, promoted rapid germination and emergence but was also found, in many cases, to increase seedling vigour, advance crop development, and increase yield.

Other benefits of on-farm seed priming have been observed. Musa *et al*. (2001) reported that priming chickpea seed for 8 h significantly reduced the damage caused by collar rot (*Sclerotium rolfsii*) in Bangladesh in two contrasting seasons. Recent work in Pakistan has demonstrated that mungbean (*Vigna radiata*) grown from seed primed in water for 8 h before sowing showed significantly fewer serious symptoms of infection by Mungbean Yellow Mosaic Virus (MYMV) than a crop established without priming (Rashid *et al*. 2004*a*). The large differences in virus-related damage were associated with significant increases in pod weight (3-fold) and grain weight (5-fold) due to priming. Rashid *et al*. (2004*b*) also observed similar differences in MYMV infection in other mungbean priming trials.

Here we investigate further these observations from field trials of enhanced resistance to disease following seed priming in an *in-vitro* investigation of pearl millet downy mildew disease. Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is a staple food crop of the semi-arid tropical regions of India and West Africa and is better adapted

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Table 1. Crops and countries in which seed priming has been tested successfully and references to where the methods used and the results obtained can be found

Values in parentheses are mean yield increases due to priming of series of trials. Instances without quoted yield increases represent situations where farmer perceptions and intentions to continue with seed priming indicate 'success'

Crop	Countries (% yield increase reported)	References
Wheat	India (5, 13, 13), Nepal (17), Pakistan (36)	Harris *et al*. (2001*b*); Rashid *et al*. (2002)
Upland rice	India (18, 40), Nigeria (77), Sierra Leone (33), Gambia (16), Ghana (57), Cameroon (39)	Harris *et al*. (1999, 2002*b*); Harris (2003)
Maize	India (8), Nepal, Pakistan (32), Zimbabwe (14)	Harris *et al*. (1999, 2001*a*, 2002*a*)
Sorghum	Botswana, Zimbabwe	Harris (1996); Chivasa *et al*. (1998, 2001)
Pearl millet	Pakistan, India	Harris and Mottram (2005)
Finger millet	India (14)	Kumar *et al*. (2002)
Chickpea	Bangladesh (47, 22), India (45, 15), Pakistan (21)	Harris *et al*. (1999); Musa *et al*. (2001); Rashid *et al*. (2002)
Mungbean	Pakistan (56)	Rashid *et al*. (2004*b*)
Cowpea	Senegal	Braconnier and Bourou (2004)

than most other cereals to regions of low rainfall and hot, sandy soils. Downy mildew disease, caused by the obligate biotroph *Sclerospora graminicola* (Sacc.) Schroet., is a major constraint to pearl millet yields (Singh *et al*. 1993; Jeger *et al*. 1998). A standard varietal screening method (Jones *et al*. 1995) was used to investigate, at CAZS-NR, the effect of seed priming on the disease resistance of pearl millet and the results are presented in this paper.

Seed priming has been particularly effective in legumes. For example, yields of chickpea (*Cicer arietinum*) and mungbean (*Vigna radiata*) were increased substantially by priming seeds for 8 h before sowing (Harris *et al*. 1999; Musa *et al*. 2001; Rashid *et al*. 2004*b*). Yield benefits were due to a combination of better crop stands and better individual plant performance. For chickpea, Musa *et al*. (2001) in Bangladesh also noted that plants grown from primed seeds had significantly more N-fixing nodules than plants grown from non-primed seeds. This effect has also been reported in cowpea (*Vigna unguiculata*) in Senegal by Braconnier and Bourou (2004).

Since distribution of native *Rhizobium* bacteria in soils is patchy and uncertain, inoculation of legumes is a general recommendation for farmers. However, not many resource-poor farmers practice it for several reasons: lack of awareness, non-availability of good-quality inoculants in the market, relatively high cost and significant risk of failure in marginal, drought-prone environments. The recommended practice is also cumbersome: making an adhesive, preparing a slurry of the *Rhizobium* culture using an adhesive, then mixing the slurry with the seed thoroughly until all the seeds are uniformly coated with culture, followed by air-drying in the shade before sowing. Consequently, resource-poor farmers in South Asia have generally failed to adopt *Rhizobium* inoculation technologies even when large growth responses can be demonstrated (Rupela *et al*. 1994). A simpler procedure would be more acceptable to farmers so, given that seed priming has proved popular, an experiment was implemented to compare the relative effectiveness of various methods of inoculating chickpea seeds with *Rhizobium* culture.

Materials and methods

Growth and yield in a range of crops

Our work on seed priming began with *in-vitro* experiments to determine the optimum priming characteristics of the most important tropical and subtropical annual crops. The results are summarised by Harris and Mottram (2005) who concluded that it is both safe and effective to soak seeds of most of the crops listed in Table 1 (and also others reported by Harris and Mottram 2005) for 8–10 h, followed by surface drying and immediate sowing. Priming seeds for longer than these 'safe limits' is risky, although rice and maize can be soaked for longer, e.g. 16–18 h, and still show benefits.

On-station and participatory, farmer-managed, on-farm trials were then used to assess the performance of seed priming in various crops and countries. Details and results of these trials may be found in the publications listed in Table 1.

Disease resistance in pearl millet

An isolate of downy mildew was collected from a downy mildew sick plot at the Hagaz Research Station, Eritrea, in 2003, as oospores in infected plant material. This was used to infect seedlings of the universally susceptible pearl millet cultivar 7042(S)-11. These plants were then used to produce fresh sporangia for experimental inoculations.

For the experiment, 10 pots of non-primed seed of pearl millet cv. 7042(S)-11 (42 seeds per 11.5-cm-diam. pot) were sown, pots watered, and placed in a plant growth chamber (12 h day at 26°C, 12 h night at 20°C). Since primed seeds (soaked for 8 h overnight in distilled water) emerge faster than non-primed seeds, they were sown 20 h later than the non-primed seeds in 10 further pots under the same conditions. Plants were inoculated at the 1- to 2-leaf stage when there was no visible difference in development stage between primed and non-primed seedlings. Inoculations using sporangial suspensions were carried out as detailed by Jones *et al*. (1995, 2001). Disease incidence was assessed in all 20 pots 14 days after inoculation. The night before assessment took place, clear plastic sheeting was used to cover all pots thus increasing humidity and inducing diseased plants to sporulate. The number of diseased plants per pot was counted and expressed as a percentage of the total number of plants per pot. Preliminary analysis showed that standard errors were not independent of the mean so percentage data

were arcsin-transformed and analysis of variance performed using the transformed data (Snedecor and Cochran 1973).

Nodulation of chickpea

This study was aimed at assessing the effect of combining *Rhizobium* inoculation and seed priming on the nodulation and growth of chickpea in pots. The experiment was conducted twice—once between 21 Nov. 2000 and Feb. 2001 and again between 19 Dec. 2001 and Mar. 2002—at ICRISAT Center, Patancheru, A.P., India. Two varieties were used: ICCV 2 (an extra-short-duration, cream seed coat coloured, *Kabuli* variety) and ICCC 37 (a wilt-resistant, brown seed coat coloured, *desi* variety). There were 6 inoculation treatments as follows.

(1) Seed priming + *Rhizobium* inoculation combined. Peat-based *Rhizobium* culture was mixed with the water used for seed priming: 1 kg seed + 1 L of water + 3.5 g peat-based inoculants of chickpea *Rhizobium* cultures (1.75 g each of IC 59 and IC 76).
(2) Seed priming followed by seed surface-drying then inoculation with *Rhizobium* mixed with a minimum quantity of water so as to make a thick paste.
(3) Seed priming followed by *Rhizobium* inoculation while the seed was still wet by mixing *Rhizobium* culture with wet seed thoroughly till all the seeds were uniformly coated.
(4) Seed priming alone (control).
(5) No seed priming but inoculated with *Rhizobium* using methyl ethyl cellulose as a sticker (general recommendation).
(6) No seed priming and no *Rhizobium* inoculation (control).

Pots (21-cm diam.) were filled with 5 kg soil/pot obtained from a field previously used to grow flooded rice. The soil was given a basal dressing of 40 kg P_2O_5/ha as single super phosphate. Each treatment × cultivar combination was replicated 6 times (3 pots per combination for sampling at flowering and 3 at maturity). Eight seeds/pot were sown and seedlings thinned 12 days after sowing to leave 3 seedlings/pot. A sample of the treated seed at sowing was analysed for viable rhizobia by serial dilution–plant infection and most probable number method using chickpea as the test legume (Toomsan *et al*. 1984). The pots were watered with tap water and maintained at 2/3 field capacity in a glasshouse at around 25°C. The plants were protected from pests by timely sprays of an appropriate insecticide when necessary.

At flowering (around 6 weeks after sowing: 5 Jan. 2001 and 2 Feb. 2002), the plants were carefully uprooted and nitrate reductase activity was measured using the method of Kumar Rao and Dart (1987). After this assay, the roots were washed, nodules separated, counted, and kept for drying in a drier maintained at 60°C. Similarly, the roots and above-ground plant biomass were dried and weighed. At maturity (7 Feb. for ICCV 2 and 27 Feb. for ICCC 37 in 2001; 22 Mar. for ICCV 2 and 23 Mar. for ICCC 37 in 2002), roots, shoots, and pods were harvested, threshed, dried, and weighed. Data were not transformed because standard errors were found to be independent of the mean. Analysis of variance was used to test the significance of main effects of year, cultivar, and treatment together with 2-way and 3-way interactions.

Results

Development and yield

On-farm seed priming was found to be effective in increasing yields in all the crops and countries listed in Table 1. Crops include both cereals and legumes and mean yield increases due to priming (values in parentheses in Table 1) include results from constituent trials that range from zero to more than 200%, with an overall average increase of around 30%. For instance, the values for upland rice in Table 1 include results from many (2929) farmers' trials in Cameroon (3 years), Ghana (3 years), Gambia (3 years), Nigeria (2 years), and Sierra Leone (4 years). Priming only failed to increase grain yield significantly in one set of trials (15 farmers in Gambia, 1999), whereas 132 farmers in Ghana in 2001 increased yield on average by 88%, although this was a drought year and included many trials in which only primed seed produced any grain at all (Harris 2003). For mungbean in Pakistan, the mean increase due to seed priming of 56% (Table 1) was calculated from 14 trials in which there were significant differences between treatments, including one in which the increase was 206% and was associated with heavy infection by MYMV (Rashid *et al*. 2004*b*). Yet priming did not increase yield significantly in a further 5 trials, although in no case did primed seed perform worse than non-primed seed.

The response of crops to seed priming is not completely consistent and there are instances where there is no response (see above). Harris *et al*. (2001*b*) reported that the percentage increase due to priming wheat varied among sites in South Asia and was generally inversely proportional to the level of management used, although the absolute values of the extra grain due to priming were quite similar. It seems reasonable to assume that seed priming substitutes to some extent for good management so that, in favourable conditions, the effect of seed priming is small. However, since negative effects of seed priming are rare (e.g. see Harris 2002), and it costs so little, the technology is of value to resource-poor farmers in marginal conditions as a form of insurance: if conditions turn out well there is no harm done but if the season is less than optimum, seed priming may mitigate some of the effects.

Disease resistance in pearl millet

The level of susceptibility in the absence of seed priming in this pearl millet cultivar was very similar to that expected from its previous performance as a control in this disease screen (data not shown), which suggests that the screen was functioning normally. Priming seeds in water for 8 h before sowing significantly reduced the incidence of downy mildew disease in seedlings of a highly susceptible cultivar from ~80% to less than 60% (Table 2).

Table 2. Percentage of plants showing symptoms of downy mildew disease

Analysis used arcsin-transformed data. Transformed means are presented (with non-transformed means in parentheses). Values for s.e.d. and l.s.d. were calculated using transformed data

Treatment	Disease incidence (%)
Seed not primed	63.5 (79.7)
Seed primed for 8 h	50.3 (58.8)
s.e.d.	3.42
l.s.d. of means (P = 0.05)	7.19

Although the screen does not allow plants to be assessed at later stages of growth, there is a high degree of correlation between performance of cultivars in the screen and their resistance to downy mildew in the field (Jones *et al*. 2002).

Nodulation in chickpea

The results of the overall analysis of variance for nodulation characteristics, acetylene reduction, and dry matter accumulation at flowering are summarised in Table 3. The main effects of year were significant for only 2 of the 6 variables, in contrast to the consistent main effects of cultivar and treatment. None of the 2-way interactions for the number of nodules per plant, nodule weight per plant, and root dry weight per plant was significant (apart from a year × treatment interaction for the number of nodules per plant), whereas all were significant for shoot dry weight per plant, total dry weight per plant, and acetylene reduction per plant (except for a cultivar × treatment interaction for total dry weight per plant). There were no significant 3-way interactions.

In contrast, the main effects of year and cultivar on the components of yield at maturity were all significant, whereas none of the 6 components were significantly affected by treatment (Table 4). There were some significant 2-way interactions involving year and cultivar, but only 2 (pod weight per plant and seed weight per plant) involving treatment. Again, there were no significant 3-way interactions.

The effects of the treatments on variables related to nodule formation and performance are summarised in Table 5. Treatments that included seed priming and *Rhizobium* inoculation (T1, T2, and T3) produced more nodules per plant in both cultivars although, within each of these 3 treatments, ICCC 37 consistently produced more nodules than did ICCV 2 (Table 5). T5 was only significantly better than T4 and T6 (i.e. treatments without added *Rhizobium*) in ICCC 37.

Priming increased the formation of nodules in both cultivars, except where no *Rhizobium* was added (T4), and this pattern of response was repeated for nodule dry mass per plant in ICCC 37 but not in ICCV 2 (Table 5). For ICCV 2, T4 and T6 produced less nodule dry mass than the other treatments and, in this case, T5 was not significantly different from the other treatments that received extra *Rhizobium*.

For nitrogenase activity (Table 5), the 2 non-*Rhizobium* treatments T4 and T6 had the lowest values in ICCV 2, although T4 (primed seed) activity was higher than T6 (non-primed seed) in ICCC 37, but not significantly so. Nodulation and N-fixation activity was not zero in T4 and T6, presumably due to natural infection from the soil. T3

Table 3. Level of significance based on comparison of *F* ratios from analysis of variance of variables measured at flowering

Y, Year; cv., cultivar; T, treatment

Variable (per plant)	Factor					
	Y	cv.	T	Y × cv.	Y × T	cv. × T
No. nodules	0.009	0.001	<0.001	n.s.	0.004	n.s.
Nodule weight	n.s.	0.009	<0.001	n.s.	n.s.	n.s.
Root dry weight	0.004	n.s.	0.008	n.s.	n.s.	n.s.
Shoot dry weight	n.s.	<0.001	0.011	<0.001	<0.001	0.044
Total dry weight	n.s.	<0.001	0.038	<0.001	<0.001	n.s.
Acetylene reduction	n.s.	<0.001	<0.001	<0.001	0.022	<0.001

n.s., Not significant.

Table 4. Level of significance based on comparison of *F* ratios from analysis of variance of variables measured at final harvest

Y, year; cv., cultivar; T, treatment

Variable (per plant)	Factor					
	Y	cv.	T	Y × cv.	Y × T	cv. × T
No. pods	<0.001	<0.001	n.s.	<0.001	n.s.	n.s.
Pod weight	<0.001	0.01	n.s.	n.s.	n.s.	0.046
Root dry weight	<0.001	<0.001	n.s.	<0.001	n.s.	n.s.
Shoot dry weight	<0.001	<0.001	n.s.	<0.001	n.s.	n.s.
Total dry weight	<0.001	<0.001	n.s.	n.s.	n.s.	n.s.
Seed weight	<0.001	<0.001	n.s.	n.s.	n.s.	0.048

n.s., Not significant.

Table 5. Effect of seed priming and *Rhizobium* inoculation on number of nodules per plant, nodule dry mass (mg/plant), and nitrogenase activity (μM C_2H_4/plant.h) of chickpea genotypes ICCV 2 and ICCC 37 grown in pots and measured at flowering

Data are means of 2001 and 2002. Within columns, i.e. treatment means, values followed by the same letter are not significantly different ($P = 0.05$)

Treatment	ICCV 2			ICCC 37		
	No. of nodules	Nodule dry mass	Nitrogenase activity	No. of nodules	Nodule dry mass	Nitrogenase activity
T1 Primed + *Rhizobium* in water	28bc	34.8a	0.467ab	60a	51.6ab	1.169b
T2 Primed (surface-dried) + *Rhizobium* as paste	52a	40.1a	0.534a	73a	48.1b	0.793cd
T3 Primed + *Rhizobium* to coat wet seeds	39ab	40.2a	0.661a	71a	60.1a	2.51a
T4 Primed alone (no *Rhizobium*)	3d	10.4b	0.134bc	2c	14.1d	0.554de
T5 Not-primed + *Rhizobium* + methyl ethyl cellulose	15cd	29.7a	0.381abc	25b	28.9c	0.808cd
T6 Not-primed, no *Rhizobium*	2d	4.3b	0.045c	3c	10.0d	0.219e
Mean	23	26.6	0.37	39	35.5	1.009
s.e.d. of means (Treatment)	8.17**	5.62**	0.1682**			
l.s.d. ($P = 0.05$)	16.47	11.32	0.3389			
s.e.d. of means (Cultivar)	4.72**	3.24**	0.0971**			
l.s.d. ($P = 0.05$)	9.51	6.54	0.1957			

** $P < 0.01$.

produced the greatest activity in both cultivars, with the other treatments showing intermediate levels of activity, although T3 differed significantly from the other treatments only for ICCC 37.

Differences in nodulation and N-fixation were not consistently reflected in plant dry mass at flowering, apart from in ICCV 2 where plants were significantly larger in T2 and T3. There were no treatment differences in ICCC 37 apart from T4 in which plants were slightly smaller than the rest (Table 6). There were no significant differences between treatments on grain yield at final harvest in either cultivar (Table 6), although in ICCC 37, T3 outperformed all other treatments and T6 was clearly the worst.

Table 6. Effect of seed priming and *Rhizobium* inoculation on plant dry mass (g/plant, measured at flowering) and grain yield (g/plant, measured at maturity) of chickpea genotypes ICCV 2 and ICCC 37 grown in pots

Data are means of 2001 and 2002. Within columns, i.e. treatment means, values followed by the same letter are not significantly different ($P = 0.05$)

Treatment	ICCV 2		ICCC 37	
	Plant dry mass	Grain yield	Plant dry mass	Grain yield
T1 Primed + *Rhizobium* in water	1.35b	2.828	1.197a	3.737
T2 Primed (surface-dried) + *Rhizobium* as paste	1.527a	2.814	1.162a	3.388
T3 Primed + *Rhizobium* to coat wet seeds	1.512a	2.718	1.26a	4.308
T4 Primed alone (no *Rhizobium*)	1.367b	2.328	1.032b	3.324
T5 Not-primed + *Rhizobium* + methyl ethyl cellulose	1.326b	2.784	1.255a	3.712
T6 Not-primed, no *Rhizobium*	1.318b	2.935	1.219a	2.558
Mean	1.4	2.735	1.188	3.505
s.e.d. of means (Treatment)	0.0569*	0.2941		
l.s.d. ($P = 0.05$)	0.1147	n.s.		
s.e.d. of means (Cultivar)	0.0329**	0.1698**		
l.s.d. ($P = 0.05$)	0.0662	0.3422		

n.s., Not significant; * $P < 0.05$; ** $P < 0.01$.

Discussion

There is a great deal of evidence that on-farm seed priming is an effective method for resource-poor farmers to use to increase yields of a range of tropical and subtropical crops growing in marginal areas (Table 1). Although there is some evidence that the initial germination response to priming is greater in crops and situations where germination is slower, and this is often correlated with seed size (Harris and Mottram 2005), priming seems to be appropriate across a wide range of cereals and legumes and can be effective in both irrigated and rainfed crops. The consensus view, of farmers and researchers, is that yield gains result from earlier, faster germination and emergence, more vigorous early growth, earlier flowering, and hastened maturity (e.g. Harris 1996, 2003; Harris *et al*. 1999, 2001*a*; Musa *et al*. 2001). In experiments where population density and yield components have been measured (e.g. Rashid *et al*. 2004*b* for mungbean; Harris *et al*. 2002*b* for upland rice), yield increases are a consequence of better stands and better individual plant performance. Benefits appear to be a consequence of more effective use of resources in dynamic and uncertain environments, often where there is intermittent

or in particular, terminal drought (Harris 2003). It is noteworthy that the largest percentage increases due to priming have often been in situations where the non-primed crop is devastated by disease (e.g. Rashid *et al*. 2004*a*, 2004*b* for mungbean) or has succumbed to severe terminal drought (Harris 2003 for upland rice).

Observations suggest, however, that there are benefits beyond those due to enhanced general vigour or changes in phenology. Musa *et al*. (2001) and Rashid *et al*. (2004*a*) have reported that crops from primed seeds showed fewer symptoms of disease and that disease-related yield losses were smaller in primed crops. It was not possible in either case to rule out the possibility that disease resistance was the result of 'escape' or avoidance of infection due to earliness or, in the case of mungbean and MYMV disease, to differential behaviour of the whitefly vector (as described for aphids by Jones 1994).

The experiment on pearl millet and downy mildew disease reported here was designed to eliminate the effect of priming-induced changes in phenology on resistance to disease. The results suggest that priming has an effect at a more fundamental, physiological level on disease resistance. Many authors, e.g. Sticher *et al*. (1997) and Métraux (2001), have reported instances of the phenomenon known as Systemic Acquired Resistance (SAR) in a range of crops. SAR is a systemic enhancement of plant resistance to disease following a localised challenge by an elicitor, which can be a microorganism, a chemical, or even a physical stress. Although it has been reported that anaerobic conditions can induce localised cell damage and the production of metabolites associated with SAR (Blokhina *et al*. 2003), we believe that this is the first report of a SAR-like response to seed priming. Work is underway to identify and quantify changes in key signalling molecules in pearl millet in response to seed priming and disease and hence to decide if SAR is responsible for this response.

The data on the effects of Rhizobia and seed priming on nodulation, nitrogen fixation, and yield are not completely consistent but it is possible to make some general conclusions. Whereas the main effects of cultivar and treatment were consistently significant at flowering, the effects of treatment had largely disappeared by maturity. Treatments particularly expected to influence grain yield, e.g. provision of additional Rhizobia, did not do so even though variables related to nodulation and nitrogen fixation had been enhanced at flowering (Tables 5 and 6). This suggests that plant nutrients, particularly nitrogen, were not limiting growth in the pots.

Although responses of ICCC 37 were generally greater overall than those of ICCV 2, both cultivars responded positively for some variables measured at flowering to the combination of seed priming and inoculation. Plants from primed seeds in the presence of added Rhizobia (T1–T3) formed significantly more nodules and had significantly larger nodule dry mass than plants that were not primed or did not receive added Rhizobia (T4–T6). The only exception to this was the nodule dry mass of ICCV 2 T4 that was smaller, but not significantly so, than T1–T3 (Table 5). This confirms the priming-related increases in nodulation of chickpea reported from the field by Musa *et al*. (2001) and shows that use of inoculum is compatible with using primed chickpea seeds. However, the effect was less clear in terms of nitrogen fixation. Although nitrogenase activity in ICCV 2 was generally greater in T1–T3 than in T4–T6, there was no clear statistical separation between these 2 groups of treatments and the pattern was even less clear for nitrogenase activity in ICCC 37 (Table 5). ICCV 2 treatments T2 and T3 produced heavier plants at flowering than T1, T4, T5, and T6, whereas in ICCC 37 only T4 plants (primed but without added *Rhizobium*) were significantly smaller than all the other treatments (Table 6). The reasons for this are not known.

Adding inoculum to the priming water (T1) was at least as effective in boosting nodulation, or more effective, than the recommended treatment (T5), although dry matter accumulation in this pot experiment was not related to degree of nodulation and suggests that N was not limiting growth. Resource-poor farmers are often reluctant to follow the recommended practice for inoculation with Rhizobia (T5) when sowing chickpea (Rupela *et al*. 1994) yet are happy to adopt on-farm seed priming (e.g. Saha 2002) because of its many benefits (Table 1). There is thus an opportunity to promote the application of *Rhizobium* through seed priming.

Conclusions

The widely observed yield benefits of on-farm seed priming are generally related to better stand establishment and changes in phenology. On-farm seed priming is simple, low-cost, and low-risk, and resource-poor farmers in marginal areas of developing countries can use it as a form of insurance to mitigate the effects of suboptimal management or adverse physical conditions. We have shown here that priming is compatible with inoculation of seeds with Rhizobia, either in the priming water (T1) or as inoculum added to primed seeds (T2 and T3). In situations where naturally occurring *Rhizobium* is limiting and levels of soil N are low, this combination can increase the efficiency of nodulation so, at least for legumes, priming can contribute to improved crop macro-nutrition. In addition, the preliminary data on disease resistance presented here suggest that some of the reported benefits of priming might be due to enhanced resistance to disease as already reported by Musa *et al*. (2001) for chickpea and Rashid *et al*. (2004*a*) for mungbean. Since phenology-related mechanisms were not implicated in the current work, it is suggested that priming might induce some form of systemic resistance but the issue has not been resolved and requires further research

to identify and measure changes in key signalling molecules in response to seed priming and disease. For a variety of reasons noted above, on-farm seed priming should be considered a valuable component of any integrated nutrient management or integrated pest management programs for marginal areas in developing countries. Once farmers have tested seed priming for themselves, adoption has been rapid (e.g. Saha 2002).

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