Journal of Biology, Agriculture and Healthcare
ISSN 2224-3208 (Paper) ISSN 2225-093X (Online)
Vol.10, No.19, 2020

www.iiste.org

Intensification of Symbiotic Performances of Grain Legumes and Rhizobia through Joint Use of Agro-inputs: Review

Mnalku A.
Ethiopian Institute of Agricultural Research, Biological and Organic Soil Fertility Management Research Program, Holeta, Ethiopia, P.O.Box: 2003

Abstract
As it holds in sub-Saharan Africa, low grain legume crop productivity has been facing Ethiopia and is often associated with reduced N_2-fixation. For low-input users, symbiotic nitrogen fixation (SNF) can be an affordable, feasible and sustainable option particularly when employed in appropriate inoculation of effective rhizobial strains to improve the productivity of legumes. Here, we review past research interventions on agro-inputs and *Rhizobium*-legume symbiosis interaction with a view of understanding the consequence on productivity of the symbiosis. First of all, information on farm-based need-to-inoculate and reinoculate legumes with rhizobia remain fundamental when benefit-oriented inoculation is thought. In addition, co-application of nutrients like phosphorus (P) or Sulphur (S), organic amendments, plant growth promoting microbes (PGPMs), arbuscular mycorrhizal fungi (AMF) and lime highly complemented rhizobia in enhancing symbiotic effectiveness. Sole lime application appeared no to improve yield of legumes, its co-application with rhizobia, acid tolerant variety and P fertilizer enhanced nitrogen (N) and grain yield of legumes. The reaction of rhizobia and symbiosis to seed-dresser pesticides may be detrimental or beneficial (indirectly) depending on the rate, and type of the pesticide and the legume. Thus, co-application of rhizobial strains and agro-inputs generally enhanced the productivity of rhizobial-legume symbiosis, but this should be done with prior refining through context-based scientific research.
Key words: Agro-input, Inoculation, Nodulation, Rhizobia, Symbiotic Effectiveness
DOI: 10.7176/JBAH/10-19-01
Publication date: October 31st 2020

Introduction

In Ethiopia grain legumes (GLs) which rank second in area coverage, production, and grain yield next to cereals, are mostly grown by private peasant holdings under rain-fed condition [1]. Cool season grain legumes such as faba bean, chickpea lentil, field pea and warm season grain legumes such as soybean, common bean, groundnut, mung bean collectively account about 13 percent of the total arable land. However, cool season grain legumes comprise area and production share of 74.4 and 77% of the total grain legume production, respectively [1]. GLs are priceless crops in terms of improving food-feed and nutrition security, income benefit, and soil fertility of the smallholder farmer's livelihoods as well as environmental safety and economic gross domestic product (GDP) growth at national level [2]. However, limited usage of modern agronomic practices in optimum integration with crop protection, nutrient management and improved variety technologies have made the productivity below the demonstrated potential in Ethiopia. About 2 and 1.4 ton of average pulse grain yield increments were reported in research and on-farm fields as compared to the national average of 2012 cropping season, respectively [3].

One amazing feature of the legumes is their endowment of bioavailable N production in association with rhizobia called SNF. GLs were estimated to fix 60-80% of their N requirement from the atmosphere [4] despite the different rates reports by different researchers. Manipulation to improve this SNF would increase plant-based protein for human consumption, increase growth and yield of subsequent crops, and reduce synthetic N fertilizer use, which highly benefits the low-input farmers. Thus, inoculating the seeds with effective rhizobial strains could be considered as practical, low cost strategy to improve SNF efficiency or grain yield [5, 6, and 7]. For this reason, rhizobia inoculants are commercialized for more than 100 years across the world and for over two decades in Ethiopia.

Despite inoculating legumes with rhizobia often display substantial improvement in nodulation, yield and soil N, the rate of improvements can be tremendously affected by the rate and method of organic, inorganic and biological agro-inputs or supplies application in the farm [8 and 9]. Several research results have been reported with regards to the interaction of variety of agro-inputs in the last five decades in Ethiopia or abroad. Hence, the general objective of this paper is to review the available research findings on rhizobia - agro-input combined applications with respect to grain yield improvement of GLs.

Need to inoculate and alternately inoculate legumes on field base

In relation to its relatively low current price and high cost of tests, farmers are often advised to use rhizobial biofertilizers in blanket particularly in most Sub-Saharan African countries. In this regard, spontaneous inoculation of legumes on farms that had no the target legume for at least five successive years was noted by [10]. Deciding whether to inoculate GLs or not, however, need to be relied on demonstrations on experimental plots. This is because the likelihood of yield response from inoculation would be higher when population of effective rhizobia is less than 20–50 cells g^{-1} soil [11]. Inoculation of high numbers of effective rhizobia to is quite essential to out-compete populations of ineffective rhizobia in the soil or build up populations that can survive limiting soil conditions [12].

The question of "how frequent a farmer need to inoculate his legume to grow on same farm?" is an important concern. The phenomenon is referred to as "alternate inoculation" and has been one of the key aspect of inoculation technology that has not yet received adequate attention by research. Rhizobia were reported to survive for over 30 years even in the complete absence its host-legume [13]. However, beyond survival, the stability of symbiotic effectiveness of inoculated strains is critical for agronomists [4]. This is because the maintenance of symbiotic effectiveness over the seasons will be predominantly constrained by the soil pH, soil temperature, soil texture, and soil water content [10]. In view of this, study reports indicated that exemption of soybean inoculation up to the second or the third growing season did not compromised yield in comparison to the successively inoculation [14 and 15].

The status of soil nutrients, soil type and strain of rhizobia are dictating factors [4, and 15]. Clay dominated soils are generally believed to better for rhizobial survival as it may give refuge in the micropores of its aggregates [16] and offer protection against protozoal grazing [17]. It is thus essential to generate customized information on the persistence and effectiveness of strains in the field environment through several seasons [4 and 18] and this would be essential in Ethiopia where biophysical diversities are tremendous [19].

Mineral nutrients and rhizobia improves SNF

By its nature, the legume-rhizobia symbiosis imposes additional requirements of calcium (Ca), potassium (K), P, boron (B), zinc (Zn), iron (Fe), manganese (Mn), cobalt (Co), sulfur (S) and molybdenum (Mo) but NO_3-N apart from what is needed for plant growth and development [20]. Absolute or relative nutrient shortage or imbalance, thus, adversely affect nodulation or SNF [21]. Inhibition of nodulation or SNF by high soil NO_3-N in all legumes is highlighted, despite its variance among species and cultivars [22]. On the other hand, legumes are generally regarded as efficient P utilizers due to soil phosphates solublization [23], finely branched root system [24], infection with mycorrhizae or adaptive growth to P limited condition like common bean [4]. However, they usually require more P than mineral N-dependent plants [25] as nodules accumulate plenty of P and nodulation cause limited root development to capture P [26]. [27] reviewed the indispensable use of 20 kg P ha^{-1} for proper SNF in Ethiopian soils. The P requirements for SNF are still species or genotype dependent (Table 1).

Table 1. Grain yield response of GLs by rhizobial inoculant (RI) and mineral nutrient supplementations in Ethiopia

Crop	Strain name**	Rate (kg ha^{-1})	Research site/condition	Yield increase (%)	Reference
Soybean	UK isolates	0.023 Fe	Shilile	2.1	[28]
	TAL-379	0.046 Fe	Shilile	6	
	MAR-1495	10P	Bako Tibe and Alaba	4	[29]
	MAR-1495	10P	Kersa and Pawe	6	[30]
	TAL-379	23N	Finote Selam	1	[31]
	Bradyrhizobia	46N	Jima	29	[32]
	MAR-1495	30S	Assosa (potted soil)	39 (Belesa-95)	[33]
		40S		46 (Wollo)	
Faba bean	Inherent rhizobia	60 P	Mekele (potted soil)	134	[34]
		15Zn		29 (ns)	
	"Local strain"	23N + 20P	Yield depleted soil	43	[35]
			Yield sustained soil	7	
	FB-1035	10P	Agarfa, Farta, Sinana, Yimana densa	13	[29]
	EAL-110	40P + 15Zn	Melkasa	24	[36]
	NM	60P + 30K	Werabe (potted soil)-shoot dry mass	60.5	[37]
Common bean	HUPvR-16	100 N	Babile and Fedis (low rhizo population)	14	[38]
		20 N	Hirna (high rhizo population	2	
	HB-429	10P	Sodo, Shalla, Dibate, Alaba, Bakotibe	10	[29]
	Phaseoli	10P	Melkasa	10 (ns)	[39]
		40P	Melkasa	39 (ns)	

Crop	Strain name**	Rate (kg ha^{-1})	Research site/condition	Yield increase (%)	Reference
Chickpea	CP-029	10P	Ada'a, Gimbichu, Demote Dale and Ginir	8	[29]
		23P	Ginir	15	[40]
			Damote gale	13	
			Ada'a	8	
	CP-M41	30S	Gondar Zuria	25	[41]

NN= not mentioned; **= Strain names are given by the researcher and often composed of the abbreviation of the institute and/or host legume, and followed by a number to indicate its history in the collection; ns = non-significant; Zn= Zinc; K= potassium; Fe = iron

The grain yield effect of RI with different nutrients namely Fe, P, N, S, Zn and K were tested on four GLs species (Table 1). In comparison to the sole inoculant, nutrient supplementation to inoculants earned grain yield increase, despite the tremendous variations in magnitude (1-134%). In fact such wide variation is expected as the studies were unharmonized and hence affected by factors of time, strain, location, even cultivars and sometimes rates with in a nutrient. Yield depleted (low-input farming systems) and low rhizobial population sites had significant increases for particularly N and P. The outputs listed hereunder except N and P are just observations that indicate the possibilities of response but are not final recommendations. Further validation of the promising treatments in integrated manner at on-farm condition and analyzing both the agronomic and the associated marginal rate of return would help reach to the ultimate verified output.

Effect of organic fertilizers on rhizobia-legumes symbiosis

Research reports from Ethiopia confirmed that co-application of rhizobia and compost showed about 23, 46 and 82% grain yield increments of faba bean over the sole RI at Haramaya, Lay Gayint and Kulumsa locations, respectively (Table 2). These relative increments are notable but still lower than the 97% soybean seed yield increase following rhizobia and 10 ton ha^{-1} vermicompost co-application over the non-composted and non-inoculated treatment reported by [42]. The nodulation and SNF capacity of cowpea also remained unchanged under 6 ton ha^{-1} vermicompost as reported by [43]. Besides, higher average nodule number per plant was reported from plants of different legume species treated with manure in relative to the control [44]. [45] also noted that soybean nodulation and SNF in low fertility soils would not be suppressed by organic amendments like vermicompost up to 148 kg N ha^{-1}. The slow N release, plant growth promotion and nutrient uptake enhancement, phosphorous contribution effects [46 and 44] as well as substrate function for rhizobia in the early infection periods would attribute to the synergy as compared to inorganic sources N.

Effect of agro-chemicals on rhizobia-legume symbiosis

Pesticides and rhizobia seed treatments are cheap insurances against seed and soil-borne pathogens, and nitrogen deficiency of pulses, respectively [47]. However, thier compatibility during joint use on pulse seeds remained controversial in the literature predominantly due to variation in methods and the lack of quantitative data [48]. Rhizobial survival reduction, poor symbiosis or poor legume performance were mentioned as major manifestations of their antagonistic effect and predominantly associated to concentration of pesticides [49].

In contrast, [50] noted the presence of compatibility potential between pesticides and RI. However, the experimental conditions [51], soil [52], strain type and climate [53], host [54], pesticide (type, concentration and time of dressing) [55] and climate determine the degree and nature of compatibility. Several studies have been made with regards to optimizing compatibility. Co-application of *Mesorhizobium* species with apron and imidalm at potted soil gave 6 and 10% chickpea shoot dry weight increment over the sole inoculated treatment. Whereas at field condition, apron and mancozeb earned 9 and 2% faba bean grain yield increment over the sole RI at Ginchi, Ethiopia (Table 2). These outputs demonstrated that not only the rate and type of the fungicide but also the sequence of dressing would have minimized the ill effects on the inoculant (Figure 1). The figure demonstrated that co-application of RI and Thiram affected both nodulation and fresh biomass of chickpea in similar trend. In terms of the degree of reduction in both parameters, Thiram then RI < RI then Thiram < RI + Thiram. It is also essential to note that rhizobia appeared to be more susceptible to fungicide at in vitro than field condition [51]. In view of this, gonotobiotic research outputs would not essentially help to predict the fate of fungicide and RI at field condition.

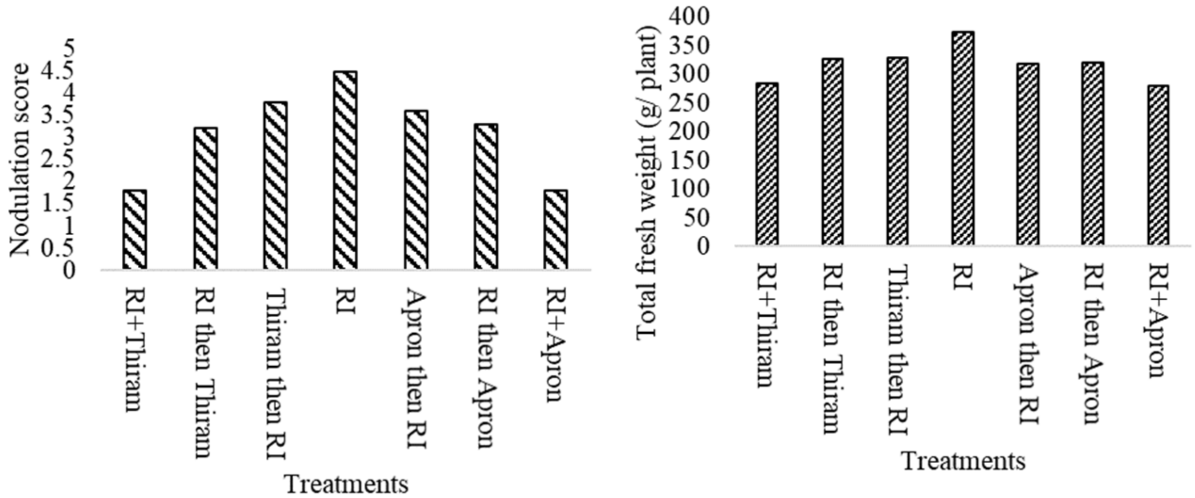

Figure 1. Effects of fungicide seed dressings on nodule score (left) and total fresh weight (right) in chickpeas adapted from [56]. (Hint: RI + Thiram= simultaneous dressing; apron then RI= sequential dressing)

Co-inoculation of RI and PGPMs

There have been an increasing number of reports on the co-inoculation of rhizobia with other plant growth promoting microbes (PGPMs) [57] or arbuscular micorrhizal fungi (AMF), enhancing a variety of legume–rhizobia symbioses in variety of synergistic mechanisms. About 40, 19 and 28% grain yield increments were reported on common bean, soybean and chickpea due to AMF and rhizobia co-inoculation, respectively [58, 59 and 60] though constraints of inoculant production are prevailing [4]. However, neither sole AMF nor co-inoculation with RI field test reports are available in cereal or legumes in Ethiopia. On the other hand, plant growth promoting rhizobacteria (PGPR) guaranteed significant improvement in the symbiotic effectiveness of RI on common bean, lentil and cowpea [43 and 61]. Field-level co-inoculation of *Mesorhizobium ciceri* (RC3) + *Bacillus* (PSB10) + *Azotobacter chroococcum* (A4) also improved seed yield of chickpea by 250% [62].

Isolation and effectiveness tastings of PGPMs are undergoing in different institutions of Ethiopia such as Addis Ababa University and Ethiopian Institute of Agricultural Research. Among these, the PGPMs test on *teff* crop at Debre Zeit during 2019 (Personal communication) was remarkably promising. As mentioned in table 2, [63 and 64] also reported encouraging synergy between RI and PGPMs, which showed about 36 and 6% grain yield increments over the uninoculated control on soybean and field pea at Assosa and Sinana, Ethiopia, respectively. The difference in the strains of rhizobia and phosphate solubilizing used as well as the site biophysical conditions were believed to contribute for the response variation. In general, selecting efficient and compatible rhizobia, AMF and PGPM strains is likely to potentially improve legumes or cereals productivity in low input farming systems or less fertile soil conditions in Ethiopia.

Table 2. Symbiotic performance of RIs in response to co-application with different farm-inputs

Crop	Experimental condition (site)	Co-applied inputs	Yield increase (%)	Reference
Soybean	Field (Assosa)	TAL-378 (RI) + PSB (*Pseudomonas*)	36 (over non inoculated)	[63]
	Field (Jima)	Bradyrhizobia + lime	30 (over sole RI)	[32]
Faba bean	Field (Haramaya)	HUFBR15 (RI) + 8 ton vermicompost (1.31% N) ha^{-1}	23(over sole RI)	[65]
	Field (Lay Gayint)	EAL-110 (RI) + 3.6 ton lime + 30 kg P ha^{-1} + 8 ton compost (0.81% N)	46	[66]
	Green house (Sand)	AWUR8 (RI) + 0.2 mL glyphosate + 0.15 g mancozeb	7 (increase in SDW over the sole RI)	[67]
	Field (Kulumsa)	EAL-110 + 9 kg N (6.2 ton ha^{-1} vermicompost	82 (over uninoculated)	[68]
	Field (Holeta)	FB-1017(RI) + apron	9 (under sole RI)	[9]
		FB-1035(RI) + mancozeb	2 (under sole RI)	
Field pea	Field (Sinana)	EAL-302 (RI) + PSB (*Bacillus*)	>3 (over 8/20 kg NP ha^{-1}	[64]
	Field (Gedo)	23/25kg NP/ha + 6 ton lime ha^{-1}		[69]

Crop	Experimental condition (site)	Co-applied inputs	Yield increase (%)	Reference
Chickpea	Green house (potted soil)	CP-029 (RI) + apron	6 (under sole I), SDW	[9]
		CP-029 (RI) + imidalm	10 (under sole I), SDW	

SDW= shoot dry weight; PSB= phosphate solubilizing bacteria

Initiatives that promote rhizobia inoculation in legume production in Sub Saharan Africa generally advise the use of balanced nutrients [70]. Though nitrate N generally inhibits SNF [71], application of micro-dose or starter N was found to be important to enhance SNF particularly in low N soils [72].

Compatibility of RI with liming

[73] estimated that close to 28.1% of the Ethiopian cultivated land is strongly acidified (pH 4.1-5.5). Such strong soil acidity highly has been constraining the production of GLs in the highlands of Ethiopia [32]. The common mechanisms are deterring survival, persistence and nodulation rhizobia, limiting SNF and stunting the growth and development of the crops are supposed to be its primary manifestations [74 and 75]. Beyond the low pH, aluminum and manganese toxicity, and P, Ca, Mg and Mo deficiency are the underlying factors [76].

Managing the deleterious effects of soil acidification through liming has long been shown to improve crop yield [77]. It is a common means of raising soil pH, which leads to greater activities of soil bacteria [78 and 79]. These activities will ultimately insure increased soil organic matter decomposition and nutrient cycling and Ca^{2+} supply [80 and 81]. However, yield increments in response to liming will not be always positive mostly due to deficiency of nutrients like P [82]. [83] also noted the negative effect of long-term use of lime on the abundance and diversity, and community structure of diazotrophs.

Integration of liming, tolerant host tolerant RI [84 and 85] and nutrients like P [86] will be highly essential to sustainably mitigate the poor productivity of GLs in highly acidic soils of Ethiopia [76]. In view of this, [87] demonstrated the improvement of SNF of cowpea by 16% through combined use of RI + lime + P compared to the un-inoculated treatment. Similarly, the use of lime (6 ton ha^{-1}) with *Bradyrhizobium* inoculation tangibly improved soybean grain yield by 30% in Jima area, Ethiopia [32]. [88] also reported a significant interaction effects of *Rhizobium*, Mo and lime on common bean in South Africa. In contrast, [86] reported the absence of nodulation improvement of cowpea in Sao Luis, Brazil via dual use of lime and inoculants.

Rhizobia potential as biocontrol agent

The plant growth promotion effect of rhizobia through SNF, nutrient solublization and phytohormone production and indirectly via pathogen growth inhibition is well documented. Other than that, there is growing evidence that rhizobia can also act as protective agents against microbial pathogens [89]. The disease suppression effects were hypothesized to be enhanced when two or more rhizobial strains are combined [90]. In this regard, [91] obtained 73% faba bean black root rot severity reduction by using composite of four rhizobial isolates at greenhouse level. In addition, sole application of *Bradyrhizobium japonicum* at field improved soybean germination by 67% and reduced *Fusarium solani*-induced foliage and root rot disease severity in 118 and 75%, respectively [92]. Besides, application of rhizobia significantly suppressed root rot fungi and root knot nematode of GLs [93].

Two remarkable points were noted in [94] from their *Rhizobium etli* inoculated common bean study against the virulent strain *Pseudomonas syringae pv. Phaseolicola*: its transgenerational (expressed down the offspring) property and non-significant reduction on symbiotic properties such as nodulation and nitrogenase activities. The later finding would give relief for the worry of agronomists that biocontrol role of rhizobia would not significant compromise SNF. The mechanisms by which the rhizobia prevent growth and development of pathogens are antagonism (production of lytic enzymes), competitive exclusion, antibiotic production, signal interference, competition for nutrients (N or Fe), hormone production, and activation of the induced systemic resistance (ISR) [95].

CONCLUSION

The use of *Rhizobium* inoculants for improvement in SNF and productivity of grain legumes has been practiced for over a century in the world and over 2 decades in Ethiopia. In those times, quite effective strains have been selected and verified for a range of GLs and use protocols have been developed. Beyond the symbionts, rhizobia survival and the symbiosis effectiveness in response to organic, inorganic and biological agro-inputs have been researched in detail. In most cases, co-application of mineral nutrients such as P, N, Mo, S, and Fe; organic fertilizers, lime and PGPMs showed positive effect on rhizobia multiplication, proper growth of the host legume, and SNF and productivity of the system. The function of inputs like lime in case of acidic soil (pH < 5.5) and P in most tropical soils remained to be fundamental. But, the co-application of lime and P tended to be an essential practice. In other hands, the PGPMs and organic fertilizer inputs were appeared to enhance symbiotic efficiency.

Though it requires context-specific observation8, the compatibility of agrochemicals and rhizobia is not always detrimental. SNF-neutral responses have been reported from several studies. Whether or not to inoculate and grow same legume species on same farm in each rotation cycle is important issue and seeks crop and situation specific research recommendations. Thus, rhizobia-legume symbiosis productivity could be enhanced sustainably through deliberate and test-based combination of agro-inputs.

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