International Journal of Environment Volume : 08| Issue : 01 | Jan.-Mar. | 2019
ISSN 2077-4505 Page:1-15

Intercropped canopies of berseem clover and barley ameliorate the forage quantity and quality beside N-transfer to the neighboured cereal

Badawi, H. Mona

Department of Microbiology, Faculty of Agriculture, Cairo University 12613, Giza, Egypt

Received: 12 Nov. 2018 / Accepted 10 Jan. 2019 / Publication date: 20 Jan. 2019

ABSTRACT

Two field experiments were conducted during the winter seasons of 2014-2015 and 2015-2016 in sandy soil to experiment the performance of berseem clover intercropped with barley for forage quality improvement. Inocula of specific *Rhizobium* for the legume and a composite of associative diazotrophs for the non-legume were applied in presence or absence of N fertilizer. Total biomass and protein yields in intercrops were greater than those of sole crops, a finding that was more obvious with the cereal where overall average increases of 187.2 and 302.9 % in dry matter and protein yields respectively were estimated. Total Land Equivalent Ratios (LERs) exceeded unity and ranged from 1.48 to 1.62 for total dry matter and from 1.28 to 1.53 for total protein. Apart from the fodder, the Ca : P ratios of untreated plants were falling in the range 1.1 : 1 - 1.3 : 1, inoculation and N fertilization modified the ratio to *ca.* 1.5 : 1, a somewhat an appropriate value for livestock. The calculated N-transferred from berseem to barley ranged from 10.17 to 57.14 kg N acre^{-1}. From this study, it is inferred that mixed cropping of berseem clover with barley gives better overall yield and income than sole culture of either crop species. In addition, the N-released from the fodder legume possibly satisfies a part of the nutrient needs of the subsequent crop.

Key words: Barley, Clover, Intercropping, Biofertilization, N-fertilization, N-transfer.

Introduction

Egyptian agriculture is confined to the Nile Valley and the Delta which represent < 3 % of total land area. However, availability of several resources, together with irrigation water all the year round and fertile alluvial soils, allow the farmers to apply various forms of agriculture intensification including relay, double and mixed intercropping systems.

The system of cultivating two crops in the same space at the same time is common among smallholder farmers (Seran and Brintha, 2010). Intercropping mostly involves cereals and legumes (Ijoyah, 2012) particularly maize-soybean, maize-cowpea, maize-groundnuts, millets-groundnuts and rice-pulses (Matusso *et al*., 2012). Indeed, intercropping is known to guarantee a more efficient use of growth factors as they capture and make a better use of radiant energy (Matusso *et al*., 2012), available water and nutrients (Sullivan, 2003), prevent pest and disease, suppress weeds, maintain and improve soil fertility (Sanginga and Woomer, 2009). This, actually, necessitates selecting the appropriate crops of varied morpho-physical nature and planning their planting geometry to reduce mutual competition for resources and enhance complementarities to increase overall productivity.

More advantages of this special cropping system can be obtained by using biological nitrogen fixation (BNF) to avoid creating environmental problems associated with the addition to soil of high quantities of mineral N fertilizers. A unique approach in this respect is the concept of "Integrated Fertilizers Management, IFM" which comprises the proper implementation of both chemical and biological fertilization.

Numerous studies (Carr *et al*., 2004; Ghosh, 2004; Ross *et al*., 2004 a,b; Campillo *et al*., 2005; Vasilakoglou and Dhima, 2008) confirmed that monocultures of legumes or cereals do not provide in most cases satisfactory results for forage production. But in intercrops, the companion cereal provides structural support for legume growth, improves light interception and facilitates mechanical harvest, while legume generally increases protein and mineral contents of the neighboured cereal.

Corresponding Author: Badawi, H. Mona, Department of Microbiology, Faculty of Agriculture, Cairo University 12613, Giza, Egypt.
E-mail: mona.badawi@cu.edu.eg, monahusseinbadawi@yahoo.com

Intercropping of berseem clover with barley should be a balanced technique for forage production (Vasilakoglou and Dhima, 2008), but this cultivation system is limited in the literature and not well studied under Egyptian conditions. Besides, the horizontal expansion that has been adopted for the last few decades to reclaim and cultivate great areas of the deserts to be added for Nile Valley and Delta encourages that every efforts have to be done to contribute in this respect. Therefore, it was decided in the present study to evaluate the yield quantity and quality of barley and berseem clover intercropped in sand soil and to what extent this cultivation system could be more productive when rational N fertilizer regimes and diazotrohic preparations are introduced into the system.

Materials and Methods

Experimental site

Two field trails were executed in a private farm at Ismailia governorate during 2014-2015 and 2015-2016 winter seasons to attain the most productive intercrop of barley and berseem clover for forage and protein yields. The experimental site lies @ 33 °E and 30 °S @ altitude of 200 m above sea level. The prevailing climate of the area is characterized by a) scant rainfall, b) relative humidity fluctuates between 40 and 60 % and c) abundance of sunshine. The average temperature and total rainfall per month along the growing seasons are given in Figure (1).

Representative soil samples were taken and analyzed adopting the procedures of Chapman and Pratt (1978). Mechanical and physico-chemical analyses revealed the following properties: coarse sand, 61.3 %; fine sand, 31.4 %; silt, 2.7 %; clay, 5.2 %, textural class, sandy; pH, 8.1; EC, 0.39 dSm^{-1}, WHC, 12.2 %; organic C, 0.08 %; total N, 0.02 %. And very limited amounts of cations and anions (meq l^{-1}): Ca^{++} (1.3), Mg^{++} (1.1), Na^{+} (1.7), SO_4^{--} (1.6), HCO_3^{-} (2.1) and Cl^{-} (0.8).

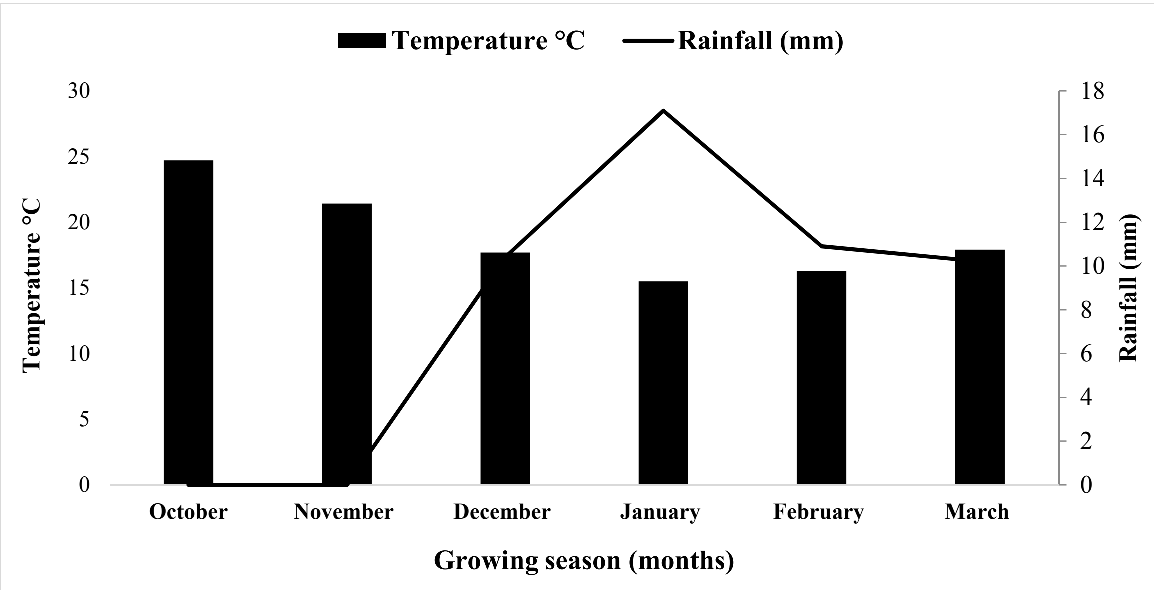

Fig. 1: Total monthly rainfall and monthly temperature during the growing season (averages of 2014-2015 and 2015-2016 seasons).

Plant materials

Seeds of berseem clover (*Trifolium alexandrium*) cv. Giza 10 and barley (*Hordum vulgar*) cv. Giza 123 were kindly supplied by the Department of Forage Research, Agricultural Research Center (ARC), Giza, Egypt.

NPK and organic fertilization

Superphosphate (P_2O_5, 15 %) and potassium sulphate (K_2O, 50 %) were incorporated into soil at the respective recommended rates of 150 and 100 kg $acre^{-1}$ during land preparation. Ammonium sulphate (N, 20.6 %) was added as 30, 60 or 120 kg N $acre^{-1}$ in successive equal doses just before seeding and after 30 and 90 days of sowing. A slow release organic fertilizer in the form of animal manure (C/N, 5 : 1) was applied at the rate of 1800 kg $acre^{-1}$.

Diazotrophs and inocula preparation

A local strain of *Rhizobium trifolii* was kindly provided by the Department of Microbiology; Soils, Water and Environment Institute, ARC. The bacterium was grown in yeast extract mannitol broth medium (Vincent, 1970) and incubated with shaking at 30 °C for 3 days. The culture was mixed at the rate of 2 : 1 (v/w) with fine peat previously neutralized with 5 % $CaCO_3$. The peat-based inoculum was adjusted to *ca.* 60 % WHC, then packed in plastic bags (100 g bag^{-1}), sealed and stored in the refrigerator. Prior to sowing, legume seeds were coated by the peat-based inoculum with Arabic gum as adhesive material and mixed uniformly. Two pioneeric multifunctional diazotrophs, *Azospirillum brasilense* and *Azotobacter chroococcum* (Badawi, 2014) characterized by high capabilities in N_2-fixation and IAA production were allowed to grow in the proper N-deficient liquid media for 3-5 days at 30 °C with continuous shaking to get a population density of *ca.* 10^8 cells ml^{-1} of each. A dual inoculant was prepared by mixing equal volumes of the individual liquid cultures, this was followed by mixing with fine peat neutralized with 5 % $CaCO_3$ (w/v) and Arabic gum. Cereal seeds were coated with this product just before planting.

Experimental layout

The experimental area was divided into 2 m x 2 m plot sizes, each comprised 10 rows spaced 20 cm apart (5 for barley alternated with 5 for berseem clover). The sward was established by direct seeding with seeding rates of 50 kg $acre^{-1}$ for barley and 30 kg $acre^{-1}$ for berseem clover.

An extra spray of each liquid inoculant was applied as 0.5 liter $plot^{-1}$. Irrespective of stand type, berseem clover was inoculated with *R. trifolii* while barley received the dual culture of associative diazotrophs *A. brasilense* and *A. chroococcum*. The water regime was sprinkling irrigation.

The layout of both field experiments was a completely randomized block design with four replicates. The experiments were planted on October 16 in 2014, October 18 in 2015 and harvested on March 24 in 2015 and March 26 in 2016. Broadleaf and grass weeds were hand-removed from plots and no serious incidence of insects or diseases were observed.

Growth parameters

In each plot, the herbage was cut, by hand at 5 cm above soil surface, three times along the growing season; 60, 120 and 160 days after seeding. Crops of the mixed cultures were harvested separately from the whole plot. The harvested samples were divided into two sets, the first was oven dried at 65 °C for 72 hr. (Vasilakoglou and Dhima, 2008) and prepared for chemical analyses and the second was dried at 70 °C to constant weight to determine the herbage dry matter yield. Total nitrogen was estimated by Kjeldahl method (Bremner, 1965). Crude protein on dry matter base was calculated by multiplying the N percentage by 6.25 (AOAC, 1980) and finally protein was expressed as protein yield per plot. Calcium and phosphorus were estimated according to Walinga *et al*. (1989). Ash content was determined at 550 °C and calculated as a percentage and then as yield (g $plot^{-1}$).

Some berseem clover plants were selected to determine number and dry weight of root nodules after careful uprooting and washing free of soil.

The relative advantage of mixed cropping compared to sole cultures was calculated for each proportion using the Land Equivalent Ratio (LER) of Mead and Willey (1980) as follows:

$$LER = \frac{Yij}{Yii} + \frac{Yji}{Yjj}$$

where: Yii and Yjj donate yields of crops i and j in sole culture while Yij and Yji are the corresponding yields in mixed cultivation.

The partial and total LERs were calculated for total biomass and nitrogen yields of both cereal and legume forage crops.

The quantities of nitrogen possibly released from berseem clover to the neighboured barley in intercropped canopies were calculated using the following adjusted equation of Hegazi and Fayez (2001):

$$\text{N-release} = \frac{1 - b/a}{c/a - 1} \text{ x}$$

where:
a, nitrogen content of non N-fertilized cereal intercropped with non N-fertilized *Rhizobium*-inoculated legume.
b, nitrogen content of non N-fertilized cereal intercropped with non N-fertilized un-inoculated legume.
c, nitrogen content of N-fertilized cereal intercropped with *Rhizobium*-inoculated legume.
x, nitrogen fertilization dose applied to plants of c.

Data analysis

Data were subjected to analysis of variance to determine significant treatment effects ($p \leq 0.05$) using Statistical Analysis System (SAS. Inst., 2000). The linear regression and coefficient of determination that express the relationship between N fertilization regime and the amounts of N transferred from the legume to the neighboured non-legume partner were calculated as well.

Results

Berseem clover emerged 3-4 days after barley emergence, however, the legume plant establishment in intercrops was not negatively affected mainly due to its high growth rate. The ANOVA for dry matter production of the solid and intercrops cut 1 for both fodder crops as well as the regrowths (cuts 2 and 3) indicated, in the majority of cases, significant differences attributed to either N fertilization or diazotroph inoculation (Table, 1). Negligible increases (< 4 %) in barley total biomass yields were attributed to inoculation of plants received no N fertilizer that observed for solid and intercropped cereal. Incorporation into soil of 30 kg N acre^{-1} significantly increased biomass yield, respective increases of 15.0 and 18.5 % were estimated for solid and intercropped canopies. Raising the N dose to 60 kg acre^{-1} exhibited more beneficial effects particularly in presence of diazotrophs where increases of 24.3 and 25.5 % were scored. Heavy N dressing with 120 kg acre^{-1} had no stimulatory influence on cereal biomass.

Table 1: Dry matter yields (g plot^{-1})* of barley and berseem clover in solid and intercropped canopies of N fertilization and diazotroph inoculation treatments

Cropping pattern	N level (kg acre^{-1})	60 DAP**		120 DAP		160 DAP		Total cuts	
		Uninoc.	Inoc.	Uninoc.	Inoc.	Uninoc.	Inoc.	Uninoc.	Inoc.
Barley									
Solid	0	179	219	299	291	99	88	577	598
	30	186	374	407	313	101	111	694	798
	60	282	343	333	418	92	118	707	879
	120	210	316	275	303	89	86	574	705
Inter-cropped	0	124	153	212	202	71	60	407	415
	30	149	302	318	257	80	89	547	648
	60	211	266	258	321	69	88	538	675
	120	141	227	199	237	71	64	411	528
Berseem clover									
Solid	0	401	542	615	767	706	734	1722	2043
	30	567	423	667	1005	602	598	1836	2026
	60	524	514	499	637	442	707	1465	1858
Inter-cropped	0	320	428	485	611	552	579	1357	1618
	30	481	338	533	804	512	502	1526	1644
	60	462	411	407	510	333	551	1202	1472
	120	500	486	401	529	366	451	1267	1466
LSD (p <0.05)		51		59		42		101	
CV (%)		19		16		21		19	

*, means of seasons 2014-2015 and 2015-2016.
** DAP, days after planting.

A percentage increase of 18.6 was attributed to *Rhizobium* inoculation of un-fertilized mono-cropped berseem clover. Nitrogen fertilization with 30 kg acre^{-1} slightly promoted dry matter production only for uninoculated plants. No more biomass was produced due to the 60 kg N acre^{-1} application. In mixed cultivation with barley, the legume responded to rhizobial inoculation to lower extent than that in solid state, average increases over uninoculated plants were 15.9 % for the former and 18.0 % for the latter.

In absence of N fertilizer, associative diazotroph inoculation of mono-cropped barley resulted in *ca.* 16 % increase in total protein yield (Table, 2). Application of 30 kg N acre^{-1} intensified the impact of inoculation with an increase of 44.9 % over uninoculated plants. Higher N levels had no obvious influence on accumulation of protein. Lower protein yields were estimated for the cereal in mixed cultivation with the neighboured legume, an observation that reported for both N-dressed plants and those kept without N.

Berseem clover greatly responded to rhizobial inoculation, either in presence or absence of mineral N. As expected, higher quantities of proteins were accumulated in the legume tissues due to N addition. The level of 30 kg N acre^{-1} was sufficient enough to support the higher protein yields, apart from rhizobia inoculation, average increases of 15.7 and 44.7 % were scored for pure and mixed stands of the legume forage respectively. Raising the N regime to 60 kg acre^{-1} had no influence on protein yields of both intercropping patterns. Irrespective of inoculation and based on the same plot area, the fodder crops in mixed canopies produced considerably high total dry matter and protein yields compared to mono-cultivation systems of either partner. Compared to unfertilized barley pure stand, as an example, the mixed cultivation resulted in 222.9 % more biomass yield (Fig. 2). Corresponding increase in total protein yield of 310.2 % was recorded as well. Raising the N fertilization rate to 30 % resulted in rather high increase in protein yield of the intercrop.

Table 2: Protein yields (g plot^{-1}) of forage crops grown in pure and mixed stands as affected by N fertilization and inoculation

Cropping pattern	N level (kg acre^{-1})	60 DAP		120 DAP		160 DAP		Total cuts	
		Uninoc.	Inoc.	Uninoc.	Inoc.	Uninoc.	Inoc.	Uninoc.	Inoc.
Barley									
Solid	0	17.5	24.4	22.5	26.3	11.3	8.8	51.3	59.5
	30	20.6	41.3	31.9	34.4	8.8	13.1	61.3	88.8
	60	30.0	27.5	26.3	43.1	6.9	13.8	63.2	84.4
	120	20.6	37.5	19.4	33.7	8.8	10.7	48.8	81.9
Inter-cropped	0	12.1	16.6	14.1	18.0	6.8	6.5	33.0	41.1
	30	14.6	30.2	23.4	26.1	6.3	9.2	44.3	65.5
	60	22.5	20.1	19.8	31.8	5.0	10.2	47.3	62.1
	120	15.0	26.3	17.5	24.4	6.9	7.6	39.4	58.3
Berseem clover									
Solid	0	71.3	93.8	88.8	140.0	101.9	109.4	262.0	343.2
	30	86.9	92.5	113.1	180.6	115.6	111.3	315.6	384.4
	60	89.1	91.4	80.6	122.5	86.3	114.4	255.7	328.3
Inter-cropped	0	43.3	66.6	51.7	81.3	72.6	66.5	167.6	214.4
	30	69.4	72.2	90.5	143.3	89.0	88.1	248.9	303.6
	60	60.6	67.5	59.4	90.2	62.8	81.8	182.8	239.5
	120	55.0	66.6	61.4	77.8	60.0	80.0	176.4	224.4
LSD (p <0.05)		**26.2**		**41.6**		**28.6**		**44.9**	
CV (%)		**22.3**		**18.4**		**19.1**		**18.5**	

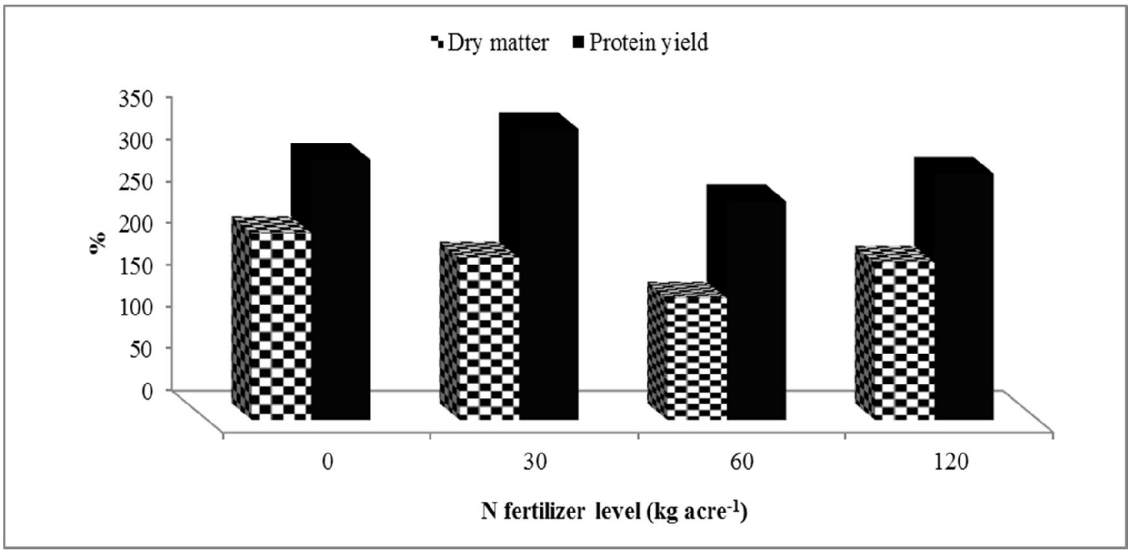

Fig. 2: Percentage increases in total biomass and protein yields of barley-berseem clover canopies over those of the cereal pure stands.

Table (3) summarizes the partial and total Land Equivalent Ratios (LERs) of the various inoculation and N fertilization treatments as well as cultivation system. In most cases, the LERs of sole barley exceeded those of berseem clover, this was reported for both biomass and protein yields. Values for mono-cropped fodders ranged from 0.69 to 0.83 and 0.63 to 0.81 for dry matter and protein yields respectively. Inoculation and/or N fertilization supported higher partial LERs for both crops. The total LERs estimates exceeded 1.0 for all the applied treatments indicating the efficiency of the cereal-legume intercropping in using the environment resources. The LER values of biomass were generally higher than the correspondings of protein yields, records of 1.48 -1.62 were calculated for the former and 1.28 - 1.53 for the latter.

Table 3: Partial and total Land Equivalent Ratios (LERs) of the various cropping, N fertilization and diazotroph inoculation treatments

Parameters / Treatments	Partial LER				Total LER	
	Barley		Berseem clover		DM	P
	DM*	P**	DM	P		
Uninoc. - Unfertil.	0.71	0.64	0.79	0.64	1.50	1.28
Inoc. - Unfertil.	0.69	0.69	0.79	0.63	1.48	1.32
Uninoc. + 30 kg N	0.79	0.72	0.83	0.79	1.62	1.51
Inoc. + 30 kg N	0.81	0.74	0.81	0.79	1.62	1.53
Uninoc. + 60 kg N	0.76	0.75	0.82	0.72	1.58	1.47
Inoc. + 60 kg N	0.77	0.74	0.79	0.73	1.56	1.47
Uninoc. + 120 kg N	0.72	0.81	-	-	-	-
Inoc. + 120 kg N	0.75	0.71	-	-	-	-

*, **; total dry matter and protein yields respectively.

Diazotroph inoculation and/or N fertilization increased calcium contents of barley although the differences in the majority of cases were statistically insignificant (Table, 4). The average nutrient yield of mono-cropped cereal exceeded that of the mixed-cultivated corresponding, percentage increases of 41.2 and 42.1 were estimated for uninoculated and inoculated plants respectively. Calcium production of berseem clover was significantly higher than that of barley. In general, *Rhizobium* inoculation supported higher calcium accumulation (7.5 - 52.1 % increase). N fertilization regimes of 60 kg acre^{-1} for barley and 30 kg acre^{-1} for berseem clover were sufficient to elevate the element pool, higher N doses had no effect in this respect. Table (5) presents the phosphorus yields of the forage crops among the different treatments. It could be noticed that the quantities of the nutrient in plant tissues generally fluctuated among the treatments in identical patterns to those of calcium.

The impact of inoculation on the legume phosphorus yields was more pronounced (5.4-41.4 %) than that for barley (5.3 - 33.7 %).

Table 4: Calcium contents (g plot^{-1}) of barley and berseem clover of cultivation systems in presence of N fertilizer and diazotroph inocula

Cropping pattern	N level (kg acre^{-1})	60 DAP		120 DAP		160 DAP		Average per cut	
		Uninoc.	Inoc.	Uninoc.	Inoc.	Uninoc.	Inoc.	Uninoc.	Inoc.
Barley									
Solid	**0**	0.85	1.18	1.56	1.46	0.39	0.28	0.93	0.97
	30	1.06	0.97	1.66	1.33	0.41	0.49	1.04	0.93
	60	1.01	1.16	1.99	2.54	0.29	0.27	1.10	1.32
	120	0.94	1.19	1.02	1.71	0.36	0.41	0.77	1.10
Inter-cropped	**0**	0.59	0.83	1.14	1.01	0.21	0.19	0.65	0.68
	30	0.72	0.74	1.22	0.90	0.29	0.32	0.74	0.65
	60	0.71	0.84	1.30	1.72	0.21	0.29	0.74	0.95
	120	0.67	0.88	0.73	1.24	0.38	0.28	0.59	0.77
Berseem clover									
Solid	**0**	4.97	6.11	5.01	8.91	6.22	7.05	5.40	7.36
	30	5.04	5.22	7.18	9.98	5.19	6.00	5.80	7.07
	60	4.01	5.67	3.94	5.33	5.85	6.91	4.60	6.00
Inter-cropped	**0**	3.21	4.11	3.44	5.12	4.22	5.61	3.62	4.95
	30	3.93	4.10	5.21	6.99	3.87	4.62	4.34	5.24
	60	3.18	4.24	3.01	4.26	3.02	5.52	3.07	4.67
	120	4.25	4.02	3.99	4.74	3.82	4.21	4.02	4.32
LSD (p <0.05)		**1.02**		**1.46**		**1.81**		**1.66**	
CV (%)		**11.71**		**19.22**		**16.61**		**21.74**	

Table 5: Phosphorus contents (g plot^{-1}) of barley and berseem clover grown in solid and intercropped systems of the different N fertilization and inoculation treatments

Cropping pattern	N level (kg acre-1)	60 DAP		120 DAP		160 DAP		Average per cut	
		Uninoc.	Inoc.	Uninoc.	Inoc.	Uninoc.	Inoc.	Uninoc.	Inoc.
Barley									
Solid	**0**	0.91	1.01	1.22	1.07	0.38	0.40	0.84	0.83
	30	0.88	0.82	1.46	2.23	0.40	0.38	0.91	1.14
	60	1.12	1.29	1.45	2.16	0.19	0.24	0.92	1.23
	120	0.98	1.11	1.66	1.41	0.28	0.37	0.97	0.96
Inter-cropped	**0**	0.62	0.74	0.81	0.79	0.28	0.26	0.57	0.60
	30	0.60	0.59	1.05	1.56	0.26	0.27	0.64	0.81
	60	0.71	0.82	1.01	1.42	0.17	0.19	0.63	0.81
	120	0.74	0.85	1.00	1.06	0.22	0.28	0.65	0.73
Berseem clover									
Solid	**0**	2.15	4.24	4.16	4.48	5.99	6.88	4.10	5.20
	30	3.42	5.99	4.27	6.00	6.06	5.66	5.58	5.88
	60	3.01	4.27	4.56	3.88	4.13	5.06	3.90	4.40
Inter-cropped	**0**	1.72	3.12	2.82	3.61	4.04	5.40	2.86	4.04
	30	2.41	4.79	3.35	4.91	4.00	3.82	3.25	4.51
	60	2.02	3.11	3.58	2.77	3.03	3.69	2.88	3.19
	120	2.20	3.98	2.86	3.11	2.10	3.06	2.39	3.38
LSD (p <0.05)		**1.3**		**2.9**		**3.8**			
CV (%)		**16.6**		**21.7**		**9.1**			

Figure (3) illustrates that the calcium: phosphorus ratios in barley tissues ranged from 0.79 : 1 to 1.2 : 1 for sole crop and 0.80 : 1 to 1.18 : 1 for mixed canopies. The respective ratios in berseem clover tissues were 1.04:1 - 1.42:1 and 1.07:1 - 1.68:1. Diazotroph inoculation, whatever the candidates, as well as N fertilization supported wider Ca : P ratios for both forages.

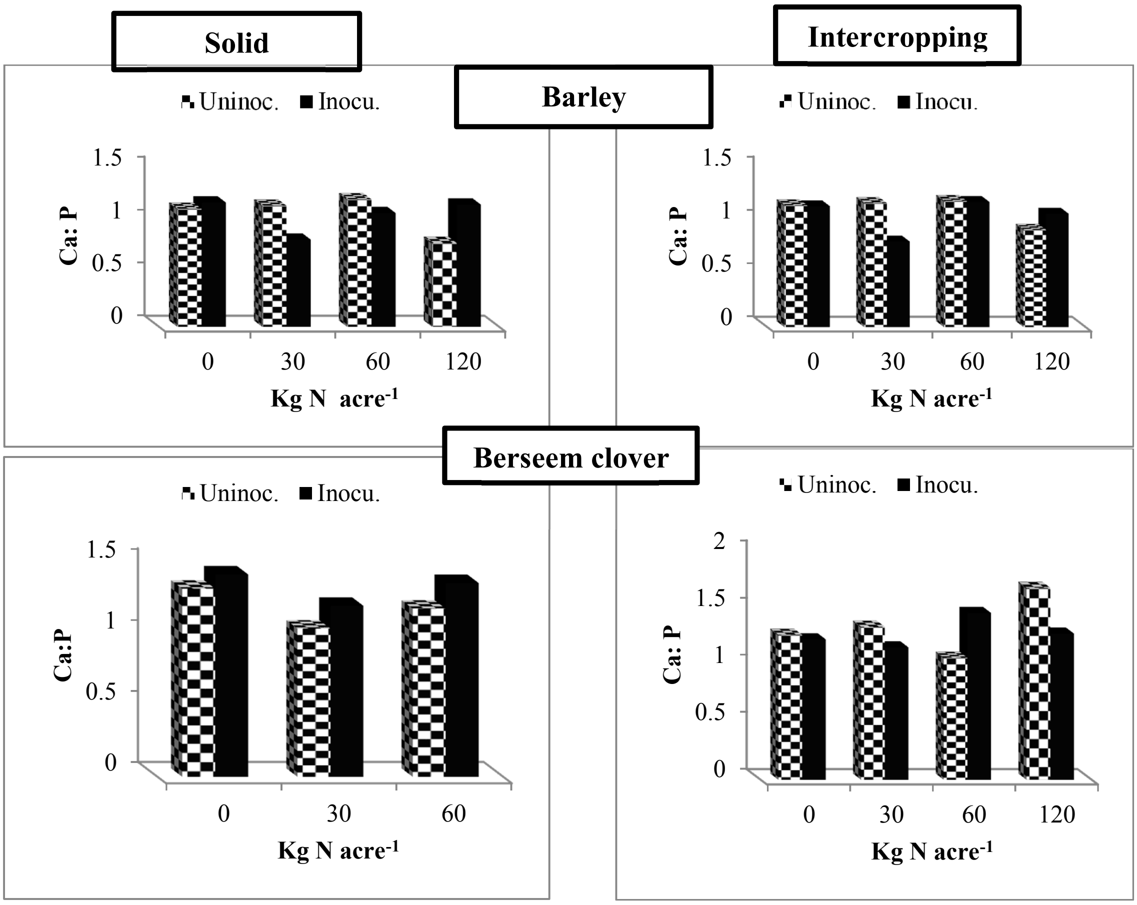

Fig. 3: Fluctuations in Ca: P ratios of the forage crop tissues among the various cultivation systems as affected by N fertilization and diazotroph inoculation.

Apart from the applied treatments, berseem clover total ash yield was > 200 % higher than that of barley. Ash contents of fodder crops were the highest for the 2nd cuts, a phenomenon that obtained for all treatments (data not shown). No significant effects on total ash yields of barley in both cropping patterns were attributed to inoculation (Fig. 4). This was not the case with berseem clover where *Rhizobium* inoculation significantly supported higher ash yields for both solid and mixed stands. The N fertilization rates of 60 kg acre^{-1} for the cereal and 30 kg acre^{-1} for the legume were the appropriates to satisfy the maxima ash yields.

Nodulation of berseem clover did positively respond to rhizobial inoculation (Fig. 5). Irrespective of N level and cultivation pattern, inoculated legume harboured considerably more nodules on their roots compared to those left without inoculation, an overall average of 21 nodules per plant was recorded for the former against 9 for the latter.

As expected, nodule formation decreased as the N fertilizer dose increased. Root nodules of inoculated plants were 15.9 - 180.4 % heavier than those of uninoculated ones. Results emphasized the superiority of mono-cropping system against intercropped one. Irrespective of inoculation and N-fertilization, the mean numbers and dry weights of nodules of the legume pure stands were 41.6 and 27.6 % higher than those of mixed ones.

The beneficial effect of intercropping on N-release from the legume to the associated non-legume was calculated based on data presented in Table (2) which represents protein yield of cultivated crops under the different experimental treatments. For example, in case of 30 kg N acre^{-1}-fertilized intercrops and applying the adjusted equation (refer to Materials and Methods), the N released from berseem to barley along the growing season is calculated as follows:

a, 41.1 g protein plot^{-1} or 6.58 kg N acre^{-1}.
b, 33.0 g protein plot^{-1} or 5.28 kg N acre^{-1}.
c, 65.5 g protein plot^{-1} or 10.48 kg N acre^{-1}.

$$\text{N-release} = \frac{1 - 5.28/6.58}{10.48/6.58 - 1} \times 30 = \frac{1 - 0.80}{1.59 - 1} \times 30 = 10.17 \text{ kg N acre}^{-1}$$

Similarly, additional N quantities of 23.53 and 57.14 kg acre^{-1} were released from the legume to the neighboured non-legume for the 60- and 120-kg N acre^{-1} dressed mixed canopies.

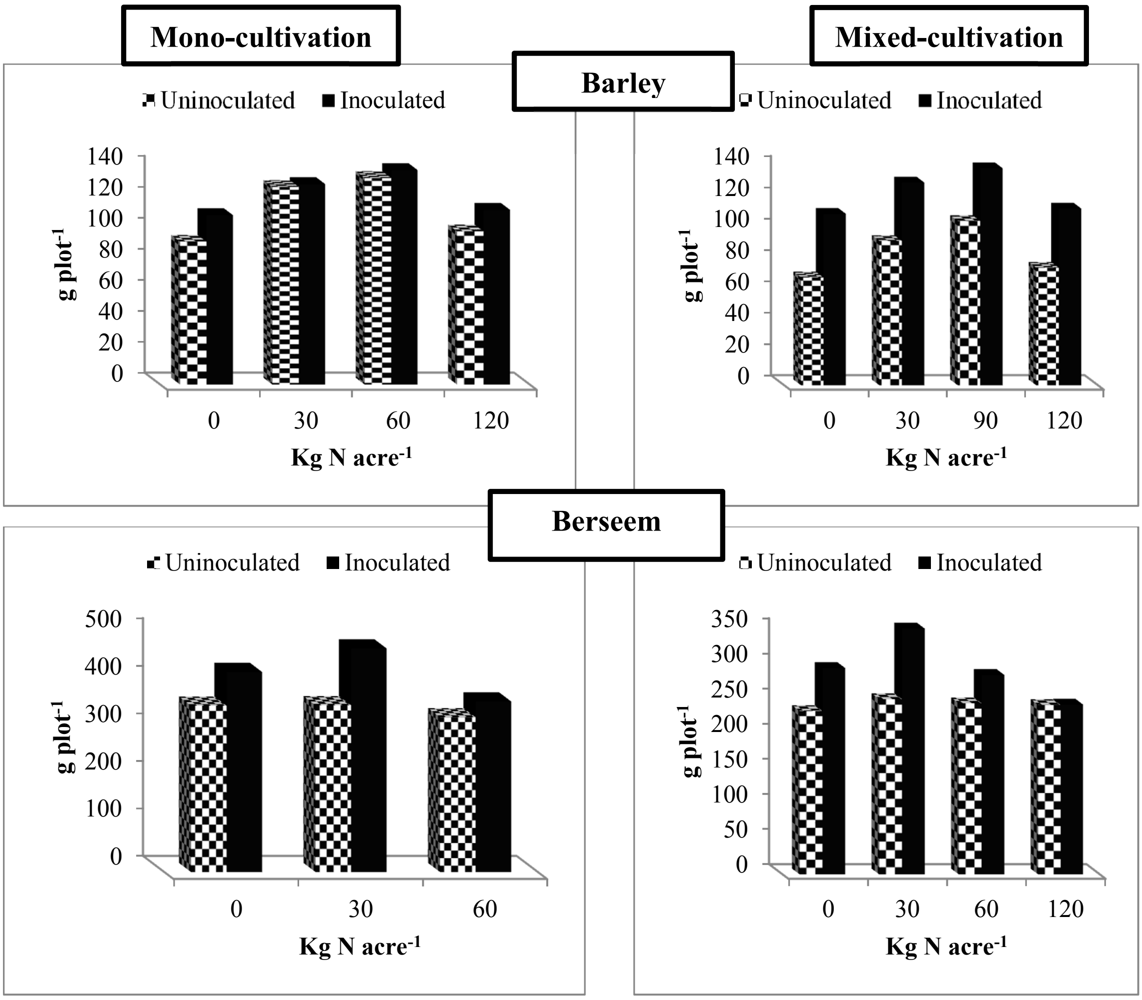

Fig. 4: Total ash contents (g plot^{-1}) of mono-and mixed-cultivated forage crops due to N fertilization and diazotroph inoculation.

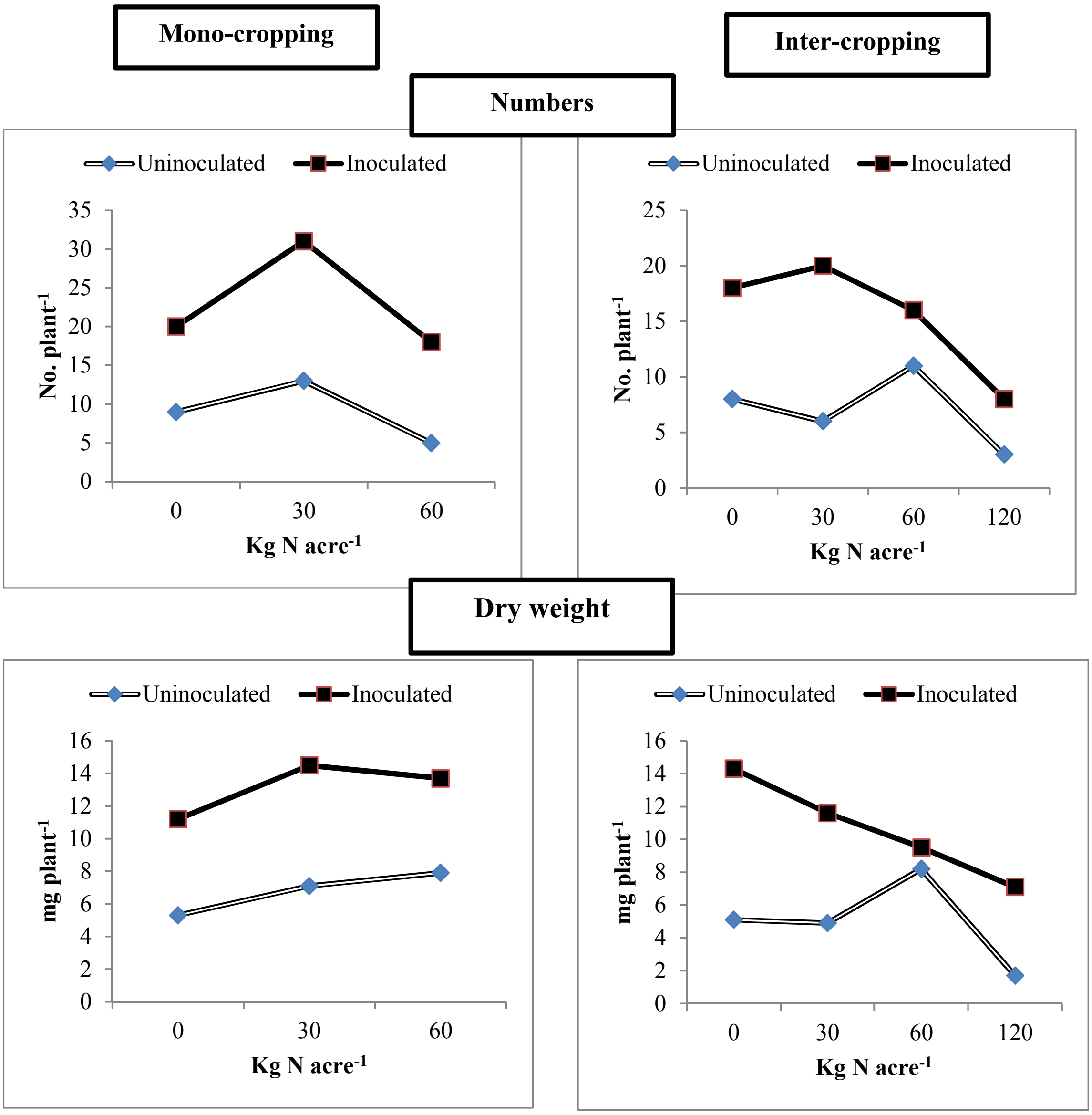

Fig. 5: Nodulation status of 90-day old solid and intercropped berseem clover as affected by N fertilization and diazotroph inoculation.

Discussion

In tropical regions, cereal/legume intercropping is practiced due to yield advantages, greater yield stability and lower risks of crop failure that are often associated with monoculture (Tsubo *et al*., 2005). A phenomenon that is more obvious in marginal soils of stressed environments. Yield increases resulting from mixed cultivation are mainly due to the presence of complimentary effects, better resource use efficiency of the mixed cultures and the buffering effects of the mixtures against diseases, weeds and abiotic factors (Agegnehu *et al*., 2006).

In Egypt, crops and cropping systems are diverse owing to large agro-ecological and cultural diversity, which in turn led to variable cropping patterns. Berseem or Egyptian clover is an annual legume of great productivity for its high growth rate and good fodder recovery after cutting. However, its forage diet could cause livestock bone abnormalities due to its incorrect Ca : P ratio and indigestible oligosaccharide content (Vasilakoglou and Dhima, 2008). Barley, on the other hand, is a profitable cereal in Egypt and is cultivated for grain and forage production. Carr *et al*. (2004)

reported that barley forage had higher digestible dry matter, lower acid detergent fiber concentration and higher crude protein content than many other cereal forages.

The present study is an attempt to secure the most appropriate conditions for harvesting great forage yield of berseem clover-barley intercropped canopies, a yield that of high nutritive value and balanced nutritive concentration in short-season environments. Actually, the extreme conditions prevailing in the experimental sites of eastern desert in Ismailia necessitates, besides heavy N dressing of up to 120 kg N acre^{-1}, the introduction of various groups of rhizospheric microorganisms (RMO) particularly those fixing N_2. Therefore, the design of the executed field trials included *Rhizobium* inoculum for the legume and another form consisting of a mixture of *Azospirillum brasilense* and *Azotobacter chroococcum* for the non-legume. Such strains proved their ability to fix N_2 and to produce plant growth regulators (PGPR) positively affecting legume (Hegazi and Fayez, 2001) and non-legume (Badawi, 2014) crops.

Earliness of booting and subsequent stages of barley than berseem clover observed in this study confirmed the results of Vasilakoglou and Dhima (2008) who found that berseem clover emerged 3 to 5 days after barley emergence. This difference in phenological events of both crops in mixture is desirable as it reduces the extent of competition and provides resource complementation. The highest agronomic yields of both forage crops were obtained by diazotroph inoculation together with the moderate level of N fertilizer. This emphasizes the fact that inoculation with N_2-fixing microorganisms does not meet all the necessary nutritional requirements of plants and simultaneous N dressing is required in such extreme conditions. Mixed cropping of the legume and non-legume favoured growth and productivity, this resulted in higher total biomass and protein yields compared to pure stand of either partner. Ross *et al*. (2004 a,b) also reported that mixed canopies of berseem clover and barley provided greater total seasonal biomass and protein yields compared to sole crops. The authors estimated increases of 63 - 180 % in total dry matter yield of the intercrops over the legume sole crop. Results of the present study showed that dry matter and protein yields of the second cut of either forage crop were greater than those at the 3rd one. These results are contradicting those of EL-Bably (2002) who found that dry matter yield of berseem clover increased from cut 3 to cut 4. The earlier planting of the legume plant during that experiment and the longer duration of the vegetative phase could be attributed to this difference (Sardana and Narwal, 2000).

Among the estimates to assess the benefits of mixed cultivation is to measure productivity using the Land Equivalent Ratio (LER), which compare the yields from growing two or more crops in the same area with yields of the same crops in monocultures. An LER greater than 1.0 indicates that intercropping is advantageous and less than 1.0 shows a disadvantage. Although both of dry matter and protein yields of the component crops in this study were low in mixtures compared to their respective sole crop yields, the total land productivity was improved in mixed cultures as supported by high LERs. The mean values of LER ranging from 1.28 to 1.62 were obtained for the mixed canopies of berseem clover and barley. This means the sole culture of each crop requires 28 to 62 % more land of the mixed crop to produce equal yields indicating greater land use efficiency of intercrops than pure stands of either. Similar results were reported for mixed cultures of lentil and barley (Kallu and Erhabor, 1990), field bean and wheat (Hauggaard-Nielsen *et al*., 2001), pea and barley (Chen *et al*., 2004), and faba bean with barley (Agegnehu *et al*., 2006).

Actually, as a cereal partner for berseem clover, this study and results of Hegazi and Fayez (2001) and Hoffmann *et al.* (2008) confirmed the superiority of barley for mixed cultivation over other cereal forages. This might be explained as a plant effect, where the deeper and more anchoring roots of barley plants might facilitate closer interaction with the root environment of the neighboured legume. Of rather interest, Shoaib *et al.* (2014) mentioned that the benefits from intercropping, on providing more dry matter and protein yields, are not limited to legume inclusion in the mixture. Sowing of two or more non-legume crops may culminate yield benefits which may rise due to structural, phonological, physiological and genetic diversity within intercrops that result in beneficial interweaving among crops and between crops and environment. These diversities reflect on exploitation of growth resources at different time and space scale (Atis *et al*., 2012). Szumigalski and Acker (2006) reported higher protein contents from wheat-canola and barley-annual rye grass mixtures than from their corresponding sole crops. This indicates that mixtures of non-legume species may also serve the purpose of forage quality improvement and this may be attributed to diversity in onset of phenological events and differential requirement of resources by the species.

It is well known that both calcium and phosphorus have a vital function in almost all tissues in the body and must be available to the livestock in appropriate quantities and ratios. The Ca : P ratios of 1 : 1 and 2 : 1 are considered the ideal for growth of livestock since this is approximately the ratio of both minerals in bone (McDowell *et al*., 1983). Mineral imbalance in soils and feed could be adjusted by applying mineral fertilizers and by exploiting genetic variability *via* breeding programs in plants for accumulation and reaction. In the present study, inoculation of forage crops with the corresponding diazotroph preparations as well as N fertilization modified the Ca : P ratio towards the proper values. Untreated fodders scored Ca : P ratios of 1.1 : 1 - 1.3 : 1, diazotroph inoculation and N application raised the ratio to 1.5:1. This ratio was modified in a number of forage crops inoculated with diazotrophs and received N fertilizer (Youssef, 1993). Modification of Ca: P in forage tissues in intercropping system of berseem clover / barley was also reported by Vasilakoglou and Dhima (2008).

Indeed, better plant development and consequently the yield of cereals mixed with legumes were taken as a clue by many authors on N-release in various cereal-legume combinations under different experimental and environmental conditions of the world including Egypt (Hegazi and Fayez, 2001; Pirhofer *et al*., 2012; Rasmussen *et al*., 2012; Lesuffleur *et al*., 2013; Chalk *et al*., 2014; Peoples *et al*., 2015; Teste *et al*., 2015 and Zhang *et al*., 2016). Those authors estimated N-release and transfer of > 90 kg N ha^{-1}. They reported considerable quantities of several nitrogenous compounds including glutamate, serine, alanine, aspartate and ammonia. In the present study, variable quantities of N-released were calculated depending upon diazotroph inoculation and N fertilization level. This indicates the compatibility and suitability of berseem clover-barley intercropped pattern under the experimental conditions. This is most probably attributed to the non-competitive type of canopy growth and development of the two studied interactants. Combined inoculation with symbiotic and associative systems maximized N-input and N-released that proportionally increased as the N fertilizer quantity increased. The relationship between N fertilization level and the quantity of N transferred from berseem clover to barley is expressed in the linear regression illustrated in Figure (6) and the coefficient of determination of $R^2 = 0.995$.

The equation adopted in the present study is recommended for computing N-release under different intercropping patterns. Recently, Zhang *et al.* (2016) came to the conclusion that direct root exudation and input from litter (both above-and below-ground) are two possible sources of N-transfer from legumes to non-legume species. They mentioned that substantial N-transfer still existed even when aboveground litter and residues are removed. And N transfer in grasses is correlated with legume root density, suggesting that fixed N was transferred mainly through root hair deposition or root exudation or *via* aboveground inputs. For instance, white clover releases high proportions of fixed N through root exudation (part of which is in the form of ammonium) and can transfer 4 % of its fixed N to neighbouring ryegrass within a two-month period (Lesuffleur *et al*., 2013).

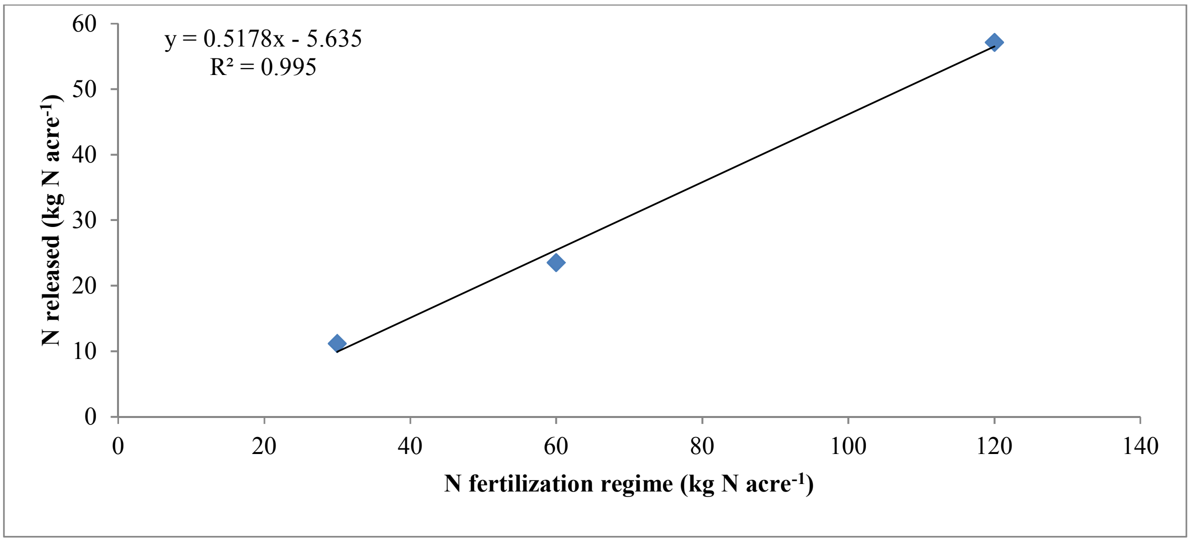

Fig. 6: Linear regression and coefficient of determination for the quantities of N-released to barley from berseem clover in intercropped canopies.

Conclusion

In Egypt, sustainable maximization of economic yields of fodder crops such as barley and berseem clover is among the cardinal goals of research and extension systems. Intercropping of both companions could be economically and environmentally promising in the Egyptian desert lands, a region characterized by somewhat low population density, small farm size and low farm income. In this study, despite the reduced barley yield due to the legume partner existence in the same area, the mixed culture as a whole exhibited higher total productivity as measured by total biomass and protein yields as well as total LERs of the two crops. The complimentary use of nutrient and water sources by the intercrop components and the need for less external inputs resulting from the cereal/ legume mixed cropping is auspicious, calling for further attention from research and development stakeholders in the desert lands. Future studies are needed, as well, to assess the extent of N capture secured from various intercropping systems particularly those in stressed environments.

Aknowledgement

The technical assistance of Dr. M. Fayez; Professor of Agricultural Microbiology, Fac. Agric., Cairo Univ. is highly appreciated.

References

Agegnehu, G., A. Ghizaw, and W. Sinebo, 2006. Yield performance and land-use efficiency of barley and faba bean mixed cropping in Ethiopian highlands. J. Europ. Agron., 25: 202-207.

AOAC, 1980. Association of Official Analytical Chemists Official Methods of Analysis, 11th ed. AOAC, Washington DC., p. 125.

Atis, I., O. Konuskan, M. Duru, H. Gozubenli, and S. Yilmaz, 2012.Effect of harvesting time on yield, composition and forage quality of some forage sorghum cultivars. Int. J. Agric. Biol., 14: 879-886.

Badawi, H. Mona, 2014. Diazotroph strain-wheat cultivar affinity guarantees proper cereal yield and soil nitrogen budget. Res. J. Agric. Biol. Sci., 10(1): 8-16.

Bremner, J.M., 1965. Total nitrogen. In: Black, C.A., *et al*. (eds.), Methods of Soil Analysis. Part 2. Agron. Monogr., ASA, Madison, WI., 9: 1149-1178.

Campillo, R., S. Urquiaga, P. Undurraga, I. Pino, and R.M. Boddey, 2005. Strategies to optimize biological nitrogen fixation in legume/ grass pastures in the southern region of Chile. Plant Soil, 273: 57-67.

Carr, P.M., R.D. Horsley, and W.W. Poland, 2004. Barley, oat and cereal-pea mixtures as dryland forages in the northern great plains. J. Agron., 96: 677-684.

Chalk, P.M., M.B. Peoples, A.M. McNeill, R.M. Boddey, M.J. Unkovich, M.J. Gardener, C.F. Silva and D. Chen, 2014. Methodologies for estimating nitrogen transfer between legumes and companion species in agro-ecosystems: a review of ^{15}N-enriched techniques. Soil Biol. Biochem., 73:10-21.

Chapman, H.D. and P.F. Pratt, 1978. Methods of Analysis for Soils, Plant and Water. Division of Agric. Sci., Univ. California, Davis, 162-165.

Chen, C., M. Westcott, K. Neill, D. Wichman, and M. Knox, 2004. Row configuration and nitrogen application for barley-pea intercropping in montana. J. Agron., 96:1730-1738.

El-Bably, A. Z., 2002. Effect of irrigation and nutrition of copper and molybdenum on Egyptian clover (*Trifolium alexandrinum* L.). J. Agron., 94: 1066-1070.

Ghosh, P. K., 2004. Growth, yield, competition and economics of groundnut/cereal fodder intercropping systems in the semi-arid tropics of India. Field Crops Res., 88: 227-237.

Hauggard-Nielson, H., P. Ambus, and E.S. Jensen, 2001. Evaluating pea and barley cultivars for complementarity in intercropping at different levels of soil N availability. Field Crops Res., 72: 185-196.

Hegazi, N. A. and M. Fayez, 2001. Biological nitrogen fixation to maximize productivity of intercropped legumes and non-legumes: Ten years of field experimentations in semi-arid deserts of Egypt. Arch. Acker-Pfl. Boden., 47: 103-131.

Hoffmann, R., T. Fabian, and F. Der, 2008. Comparison of yields and nutritive value of different spring green forage mixtures. Acta, Agric. Slovenica, Suppl., 2:143-148.

Ijoyah, M. O., 2012. Review of intercropping research on cereal-vegetable based cropping system. Sci. J. Crop Sci., 1(3): 55-62.

Kallu, B.A. and P.O. Erhabor, 1990. Barley, lentil and flax yield under different intercropping systems. J. Agron., 82: 1066-1068.

Lesuffleur, F., C. Salon, C. Jeudy, and J.B. Cliquet, 2013. Use of a ^{15}N-labeling technique to estimate exudation by white clover and transfer to companion ryegrass of symbiotically fixed N. Plant Soil., 369:187-197.

Matusso, J.M.M., J. N. Mugwe, and M. Mucheru-Muna, 2012.Potential role of cereal-legume intercropping systems in integrated soil fertility management in smallholder farming systems of sub-Saharan Africa Research Application Summary. Third Ruforum Biennial Meeting 24-28 September 2012, Entebbe, Uganda.

McDowell, L.R., J.H. Conrad, G.L. Ellis, and L.K. Loosli, 1983. Minerals for grazing ruminants in tropical regions. Extension Bulletin Anim. Sci. Dept., Univ. Florida.

Mead, R. and R.W. Willey, 1980. The concept of a "Land Equivalent Ratio'' and advantages in yields from intercropping. Expl. Agric., 16: 217-228.

Peoples, M.B., P.M. Chalk, M.J. Unkovich, and R.M. Boddey, 2015. Can differences in ^{15}N-natural abundance be used to quantify the transfer of nitrogen from legumes to neighbouring non-legume plant species? Soil Biol. Biochem., 87:97-109.

Pirhofer, W.K., J. Rasmussen, H.J. Hogh, J. Eriksen, K. Soegaard, and J. Rasmussen, 2012. Nitrogen transfer from forage legumes to nine neighbouring plants in a multi-species grassland. Plant Soil, 350: 71-84.

Rasmussen, J., R. L. Gylfadóttir, J. Eriksen, and A. Helgadóttir, 2012. Spatial and temporal variations in N transfer in grass-white clover mixtures at three Northern European field sites. Soil Biol. Biochem., 57:654-662.

Ross, S.M., J.R. King, J.T. O'Donovan, and D. Spaner, 2004 a. Forage potential of intercropping berseem clover with barley, oat, or triticale. J. Agron., 96:1013-1020.

Ross, S.M., J.R. King, J.T. O'Donovan, and D. Spaner, 2004 b. Intercropping berseem clover with barley and oat for forage. J. Agron., 96:1719-1729.

Sardana, V. and S.S. Narwal, 2000. Influence of time of sowing and last cut for fodder on the fodder and seed yields of Egyptian clover. J. Agric. Sci., 134:285-291.

Sanginga, N. and P. L. Woomer, 2009. Integrated soil fertility management in Africa: Principles, Practices and Development Process. (Eds.). Tropical Soil Biol. Fertility. Inst. Inter. Centre Tropical Agric. Nairobi.

SAS Inst, 2000. SAS/STAT user's guide. Version 8e. SAS Inst., Cary, NC.

Seran, T. H. and I. Brintha, 2010. Review on maize based intercropping. J. Agron., 9(3): 135-145.

Shoaib, M., M. Ayub, M. Shehzad, N. Aktar, M. Tahir, and M. Arif, 2014. Dry matter yield and forage quality of oat, barley and canola mixture. Pak. J. Agric. Sci., 51(2):433-439.

Sullivan, P., 2003. Intercropping principles and production practices. Appropriate Technology Transfer for Rural Areas Publication. Retrieved from http://www.attra.ncat.org.

Szumigalski, A.R. and R.C.V. Acker, 2006. Nitrogen yield and land use efficiency in annual sole crops and intercrops. J. Agron., 98:1030-1040.

Teste, F.P., E.J. Veneklaas, K.W. Dixon, and H. Lambers, 2015. Is nitrogen transfer between plants enhanced by contrasting nutrient-acquisition strategies? Plant Cell Environ., 38:50-60.

Tsubo, M., S. Walker, and H.O. Ogindo, 2005. A simulation model of cereal-legume intercropping systems for semi-arid regions II. Model application. Field Crops Res., 93:23-33.

Vasilakoglou, I. and K. Dhima, 2008. Forage yield and competition indices of berseem clover intercropped with barley. J. Agron., 100(6): 1749-1756.

Vincent, J.M., 1970. A Manual for the Practical Study of Root Nodule Bacteria. International Biological Programme (IBP) Hand book No. 15 Blackwell Scientific Publications, Oxford.

Youssef, H. Hanan, 1993. Improving efficiency of nitrogen fixing bacteria associated with field crops. M.Sc. Thesis, Fac. Agric., Cairo Univ., Giza, Egypt.

Walinga, I., W. Van Vark, V.J.G. Houba, and J.J. Van der Lee, 1989. Plant analysis procedures (Soil and Plant Analysis, Part 7). Department of Soil Science and Plant Nutrition, Wageningen Agric. Univ., 62-66; 138-142.

Zhang, H.Y., Q. Yu, X.T. Lu, S. E. Trumbore, J.J. Yang, and X.G. Han, 2016. Impacts of leguminous shrub encroachment on neighbouring grasses include transfer of fixed nitrogen. Oecologia, 180: 1213-1222.