Advantages of Intercropping Soybean with Maize Under Two Maize Plant Distributions and Three Mineral Nitrogen Fertilizer Rates

Moshira Ahmed El-Shamy¹, Tamer Ibrahim Abdel-Wahab¹, Sherif Ibrahim Abdel-Wahab¹, Samuel Berty Ragheb²

¹Crop Intensification Research Department, Field Crops Research Institute, Agricultural Research Center, Giza, Egypt

²Food Legumes Research Department, Field Crops Research Institute, Agricultural Research Center, Giza, Egypt

Email address:

moshira_shamy@yahoo.com (M. A. El-Shamy)

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Abstract: The advent of further bioengineering for growing soybean with maize holds only promise for the future of the crop intensification field. The present research studied responses of maize crop and its yield attributes to decrease mineral nitrogen (N) inputs by growing soybean with two maize plant distributions under three cropping systems. Local maize variety T.W.C. 310 was grown under intercropping and sole cultures in one row/ridge in one and two plants/hill spaced at 30 and 60cm, respectively, that received three mineral N fertilizer rates (4, 5 and 6 g N/plant), while local variety of soybean seeds Giza 82 were drilled in two rows/ridge. A split – split – plot design with three replications was used. Light intensity within maize canopy, ear leaf N and indole acetic acid (IAA) contents were affected by all the studied factors. Mixed pattern increased grain yields per plant and per ha by 1.56 and 3.98 %, respectively, in comparison with sole culture in addition to yielding 1.74 ton/ha of soybean. Increasing number of plants from one to two plants/hill by increasing plant spacing from 30 to 60 cm increased grain yields per plant and per ha. There were no significant differences between the medium and the highest mineral N fertilizer rates for all the studied traits. Soybean improved N use efficiency (NUE) for maize plant of mixed pattern. The mixed pattern had a total yield increase of 29.79 % than sole maize. Growing soybean on both sides of maize ridge that distributed to two plants/hill spaced at 60 cm decreased 47.6 kg N/ha of the recommended mineral N rate of maize plants which formed the best bioengineered treatment under Egyptian conditions.

Keywords: Bioengineering, Soybean, Maize Plant Distributions, Mineral N Fertilizer, IAA, NUE

1. Introduction

Bioengineering as a defined field is relatively new, although attempts to solve biological problems have persisted throughout history. Recently, the practice of bioengineering has expanded beyond large-scale efforts for agricultural advancement. The highest maize (*Zea mays* L.) yield productivity depended on soil fertility, maize variety and nitrogen (N) fertilization (Ding *et al.*, 2005). Maize plant development is strongly dependent on the availability of N in the soil and the efficiency of N utilization for biomass production and yield (Sonnewald, 2012). It is the world's most widely grown cereal and produced cereal crops (FAO, 2013). It is an important crop grown in summer/autumn throughout

Egypt for both grain and green fodder purposes. The demand for mineral N fertilizers that required for maize growth and development has prompted to minimize the N – fertilizer rates, especially N – the most frequent limiting factor of intensive agriculture production – which is extremely costly for many farmers (Montanez and Margarita, 2013). In addition, large losses of reactive N to the environment are still common from agricultural systems through transport of nitrate to ground water or surface waters and emissions of nitrous gases to the atmosphere (Davidson *et al.*, 2014).

The most studied and longest exploited plant growth promoting rhizobacteria are the rhizobia for their ability to fix atmospheric N (Werner, 1992) in association with their legume host plants. Most of the research to optimize symbiotic N fixation and to increase the use of legumes in cropping

systems has been in part stimulated by increasing fertilizer prices and environmental concerns (Sanginga *et al.*, 2003). There has been increasing evidence that rhizobia can play an important role in the growth promotion of non – legumes (Lupwayi *et al.*, 2004). The subsidiary effect of having an N – fixing legume in a cropping system has been shown to be an important resource for N for intercropped cereal (Shen and Chu, 2004). In response to changes in N supply, plants display elaborate responses at both physiological and morphological levels to adjust their growth and development (Lošák *et al.*, 2010). In grass crops, the transfer of the fixed N to the plant occurs slowly and only a small amount becomes available to plant; therefore, biological N fixation (BNF) by these bacteria (*Azospirillum brasilense* and *Herbaspirillum seropedicae*) in association with grass crops is only able to meet plant N demand partially (Hungria *et al.*, 2011).

Plant hormones are chemical messengers that are produced in one part of the plant and have a physiological effect on a target tissue that may be distant from the site of production. Auxins, cytokinins, gibberellins, abscisic acid, and ethylene are the best known plant hormones. Natural and synthetic auxins have been used extensively in plant cell, tissue, and organ cultures to elicit specific morphogenetic responses (Bhojwani and Razdan, 1983). Until recently, the biosynthesis of indol-3-acetic acid (IAA "auxin") was hypothesized to occur via oxidative decarboxylation and deamination of the amino acid tryptophan (Bandurski *et al.*, 1995). Since plant meristems are actively growing tissues where cell division governs sink strength, environmental signals can modulate plant responses to the growing conditions through changes in phytohormone concentrations, thus controlling assimilate partitioning between different sink tissues (Hartig and Beck, 2006). Amongst phytohormones, IAA was closely linked to N signaling (Kiba *et al.*, 2011).

Obviously, plant responses to nutrient availability depend on the availability of other required resources. Solar radiation must be immediately intercepted by maize plant in order for it to drive photosynthesis. The light inhibition of IAA transport was closely related to the increase of IAA immobilization (Kondo *et al.*, 1969). Certainly, light influences ammonia assimilation at the glutamine synthetase reaction in maize

leaves (Klaus *et al.*, 1985), especially leaf N directly affects net photosynthetic rates (Meziane and Shipley, 2001).

Also, plant spacing varies with the growth of plants and the growing environments (Zhao *et al.*, 1997). Doubling maize number from two to four plants/hill by increasing spacing between plants from 30 to 60 cm resulted in significant increments in maize yield and its attributes under intercropping and sole culture (Metwally *et al.*, 2009). Therefore, the main objective of the present research was to study responses of maize crop and its yield attributes to decrease mineral N inputs by growing soybean with two maize plant distributions under three cropping systems.

2. Materials and Methods

A research was carried out at Sakha Agricultural Experiments and Research Station, Agricultural Research Center (A.R.C.), Kafr El – Sheikh governorate (31°06'42"N, 30°56'45"E, 17 m a.s.l.), Egypt, during 2012 and 2013 summer seasons. Wheat was the preceding winter crop in both seasons. Bioengineering approach was the interaction among cropping systems, maize plant distributions and mineral N fertilizer rates in addition to varieties of both species and environmental (climatic and edaphic) conditions such as temperature, relative humidity, sunshine hours, water duty, mechanical, chemical and biological soil structure. Mechanical analysis of the soil (0 – 30 cm), sand (9.5 %), silt (53.0 %), clay (37.5 %), the experimental soil was a loamy clay texture. Chemical analysis of the soil (0 – 30 cm), pH value (7.95), organic matter (1.64 %), available N (30.5 ppm), available phosphorus (7.77 ppm) and available potassium (392.5 ppm) were analyzed by Water and Soil Research Institute, A.R.C. Methods of mechanical and chemical analysis were described by Chapman and Pratt (1961). The average monthly temperature for the two years ranged from 20.8 to 34.7 °C in the first season and from 21.8 to 33.8 °C in the second season, the average relative humidity ranged from 50.0 to 84.9 % in the first season and from 45.7 to 83.6 % in the second season, meanwhile the average sunshine hours ranged from 10.4 to 12.5 hours (Table 1).

Table 1. Meteorological information data of Kafr El-Sheikh governorate (May–September) in 2012 and 2013 summer seasons.

Season	2012					2013				
	Temperature (°C)		Relative humidity (%)		Sunshine (hours)	Temperature (°C)		Relative humidity (%)		Sunshine (hours)
	Max.	Min.	7.30	1.30		Max.	Min.	7.30	1.30	
May	30.8	20.8	75.7	50.0	11.0	31.4	21.8	75.0	45.7	11.0
June	33.6	23.5	79.6	50.7	12.5	32.4	23.9	74.6	51.2	12.5
July	33.2	25.3	84.0	53.0	12.3	32.3	24.3	79.5	54.7	12.3
August	34.7	25.0	84.9	52.1	11.5	33.8	24.8	83.6	60.5	11.5
September	32.3	22.7	82.8	52.3	10.4	32.5	22.9	81.0	56.6	10.4

- Rice Research and Training Center (RRTC), Sakha Agriculture Research Station, Monthly weather observations.
- Average relative humidity (%), at 7.30 and 13.30 hour.

Calcium super phosphate (15.5 % P₂O₅) at rate of 357 kg / ha and potassium sulfate (48.0 % K₂O) at rate of 119 kg / ha were applied during soil preparation for planting maize and soybean in the two summer seasons. Water was supplied by

furrow irrigation (water duty = 8806 m³ / ha in 2012 summer season and 8330 m³ / ha in 2013 summer season). Seeds of the early – maturing soybean variety Giza82 were inoculated with *Bradyrhizobium japonicum* and gum Arabic was used as

a sticking agent. Soybean seeds were sown on 9th and 13th May in 2012 and 2013 seasons, respectively, meanwhile, maize variety T.W.C.310 was sown 15 days later. Soybean was thinned to two plants spaced at 15cm. Soybean plants were harvested on 14th and 20th August at 2012 and 2013, respectively. Maize plants were harvested on 23rd and 26th September at 2012 and 2013, respectively. The experiment included three cropping systems (2:2 pattern, mixed pattern and sole culture) and two plant distributions (one plant/hill spaced at 30 cm and two plants/hill spaced at 60 cm) that received three mineral N fertilizer 'ammonium nitrate, 33.5 % N unit' rates (190.4, 238.0 and 285.6 kg N/ha was expressed as 4, 5 and 6 g N/plant). Figure (1) shows different cropping systems with two plant distributions as follows:

1. Two maize ridges alternating with another two of soybean. Maize was grown in one row/ridge with leaving one plant/hill spaced at 30 cm or leaving two plants/hill spaced at 60cm. Soybean seeds were drilled in two rows/ridge. This pattern resulted in 23.800 and 190.400 plants of maize and soybean / ha, respectively.
2. Mixed pattern, maize was grown in one row/ridge with leaving one plant/hill spaced at 30 cm or leaving two plants/hills spaced at 60 cm, meanwhile soybean seeds were drilled in both sides of maize ridge. This pattern resulted in 47.600 and 380.800 plants of maize and soybean / ha, respectively.
3. Sole maize: pure stand of maize ridges by growing one plant/hill spaced at 30 cm (recommended pattern) or growing two plants/hill spaced at 60cm. This pattern resulted in 47.600 maize plants/ha.

In addition to sole soybean by drilling soybean seeds in two rows/ridge. This pattern resulted in 380.800 plants of soybean/ha (this is recommended pattern).

A split – split – plot design with three replications was used. Cropping systems were randomly assigned to the main plots, maize plant distributions were allotted in subplots and mineral N fertilizer rates were devoted to sub – sub - plots. The area of sub – sub – plot was 16.8 m², it consisted of four ridges, and each ridge was 6.0 m in length and 0.7 m in width.

At 75 days from maize sowing, light intensity (lux) inside maize canopy at middle and bottom of the plant was recorded by Lux-meter apparatus at mid-day on five plants from each plot and expressed as percentage from light intensity (100%) measured above the plants. Also, the following variables were recorded as analyzed by the General Organization for Agricultural Equalization Fund, Agricultural Research Center, Giza, Egypt and the Regional Center for Food & Feed, A.R.C., Giza, Egypt:

- Total count of rhizobia in rhizosphere of maize roots (colony forming unit 'cfu'). The culture medium was yeast extract mannitol agar, counting method was done by dilution plate count, incubation condition was 30 °C/2 – 3 days. Methods of microbial analysis were described by Alexander and Clark (1965).
- Ear leaf N content (mg/g).
- Free IAA content of ear leaf (ppm). Methods of analysis were described by Hutzinger and Zander (1969).

At harvest, the following traits were measured on ten guarded plants from each plot: Plant height (cm) and ear leaf area (cm²) was determined as leaf length x leaf width x 0.75 according to Francis *et al.* (1969). Other traits recorded were number of ears / plant, ear weight (g), 100 – grain weight (g) and grain yield per plant(g). Maize grain and soybean seed yields / ha (ton) were recorded on the basis of experimental plot area by harvesting all plants of each plot. Maize grain and soybean seed yields / ha formed total yield / ha (ton).

N use efficiency (NUE) for each treatment was determined using the agronomic efficiency (AE) and partial factor productivity (PFP) indices (Dobermann, 2007): $AE = (Y - Y_0) / F$ and $PFP = (Y_0 / F) + AE$, where F = amount of (fertilizer) nutrient applied (kg/ha); Y = Crop yield with applied nutrients (kg/ha) and Y₀ = crop yield (kg/ha) in a control treatment with no N. A basic assumption was that N uptake is the same in fertilized and unfertilized plots.

This assumption was made with a caution since soil N transformations and root development may differ between fertilized and unfertilized plots (Weber and Day, 1996; Brye *et al.*, 2002).

Analysis of variance of the obtained results of each season was performed. The homogeneity test was conducted of error mean squares and accordingly, the combined analysis of the two experimental seasons was carried out. The measured variables were analyzed by ANOVA using MSTAT-C statistical package (Freed, 1991). Mean comparisons were done using least significant differences (L.S.D) method at 5 % level of probability to compare differences between the means (Gomez and Gomez, 1984).

3. Results and Discussion

Light intensity within canopy, ear leaf N and IAA contents at 75 days from maize sowing

Cropping systems

Light intensity at middle and bottom of maize plant was affected by the cropping systems (Table 2). Maize plant of 2:2 pattern had the highest light intensity at middle and bottom of maize plant compared to that of the other patterns. Alternating ridges 2:2 pattern increased ($P \leq 0.05$) light intensity at middle and bottom of the plant by 13.45 and 30.73 percent, respectively, compared to mixed pattern. Also, this pattern increased significantly light intensity at middle and bottom of the plant by 13.45 and 30.45 percent, respectively, compared to sole culture. Clearly, light interception of mixed pattern was statistically equal ($P > 0.05$) to those of sole culture. These data indicate that spatial arrangement of mixed pattern was identical for spatial arrangement of sole culture because it had the same plant density/unit area. These results show that light transmission inside canopy of the plant was not changed between mixed and sole cultures.

On the other hand, ear leaf N content was affected significantly by cropping systems (Figure 2). Maize plant of 2:2 pattern had higher ear leaf N content than that of sole culture. This pattern increased ($P \leq 0.05$) ear leaf N content by 8.74 percent compared to sole culture.

Figure 1. Intercropping soybean with maize and sole maize under two plant distributions.

Figure 1. Continued.

Figure 1. Continued.

Table 2. Effect of cropping systems, maize plant distributions, mineral N fertilizer and their interactions on light intensity within maize canopy, combined data across 2012 and 2013 seasons.

Cropping system	Maize plant distributions	Percentages of light intensity at							
		Middle of the plant				Bottom of the plant			
		4 g N /plant	5 g N /plant	6 g N /plant	Mean	4 g N /plant	5 g N /plant	6 g N /plant	Mean
2:2 pattern	1P/30cm	26.52	26.02	25.96	26.16	12.11	11.88	11.73	11.90
	2P/60cm	27.27	26.55	26.60	26.80	12.93	12.41	12.46	12.60
	Mean	26.89	26.28	26.28	26.48	12.52	12.14	12.09	12.25
Mixed pattern	1P/30cm	23.39	22.61	22.66	22.88	9.31	8.85	8.89	9.01
	2P/60cm	24.15	23.71	23.59	23.81	10.10	9.58	9.55	9.74
	Mean	23.77	23.16	23.12	23.34	9.70	9.21	9.22	9.37
Average of intercropping		25.33	24.72	24.70	24.91	11.11	10.67	10.65	10.81
Sole culture	1P/30cm	23.44	22.58	22.70	22.90	9.37	8.93	8.99	9.09
	2P/60cm	24.08	23.68	23.63	23.79	10.02	9.52	9.56	9.70
	Mean	23.76	23.13	23.16	23.34	9.69	9.22	9.27	9.39
Average of maize plant distributions	1P/30cm	24.45	23.73	23.77	23.98	10.26	9.88	9.87	10.00
	2P/60cm	25.16	24.64	24.60	24.80	11.01	10.50	10.52	10.67
Average of N fertilizer rates		24.80	24.19	24.18	24.39	10.63	10.19	10.19	10.33
L.S.D.0.05 Cropping systems (C)					1.12				0.77
L.S.D.0.05 Maize plant distributions (D)					0.71				0.59
L.S.D.0.05 Mineral N fertilizer (N)					0.52				0.38
L.S.D.0.05 C x D					1.26				0.81
L.S.D.0.05 C x N					1.33				0.84
L.S.D.0.05 N x D					0.94				0.67
L.S.D.0.05 C x D x N					1.55				0.92

These results could be due to spatial arrangement of 2:2 pattern formed 50:50 percent of number of each crop/unit area compared to sole crops. Consequently, this pattern decreased intra-specific competition between maize plants for basic growth resources (especially solar radiation transmission) which enhanced efficiency of photosynthesis process in maize plant. It is known that the light environment surrounding plants affects seedling growth (Schopfer, 1984). In vegetative canopies, the profile of N concentration in leaves has been shown to be positively correlated to the light environment experienced by the leaves (Werger and Hirose, 1991). The N concentration in a leaf layer can be related to the proportion of the incoming light that reaches that layer (Milroy *et al.*, 2001). Moreover, ribulose 1,5 carboxylase / oxygenase (Rubisco) activity and phosphor enol pyruvate carboxylase (PEPc) activity that plays a major role in photosynthesis process varies with leaf age, N content and light environment during the growth of plants (Tazoe *et al.*, 2006).

Also, free IAA content of ear leaf was affected significantly by cropping systems (Figure2). Free IAA content of ear leaf in maize plant of 2:2 pattern was decreased ($P \leq 0.05$) by 7.05 percent compared to that of sole culture. These results reveal that vegetative growth and development of the plant benefited greatly from available environmental climatic resources especially solar radiation where light has been shown to alter the levels of IAA (Behringer and Davies, 1992). Resource use efficiency is not likely to be much affected in intercropping systems with component crops that differ in growing period, since competition between component crops is weak (Fukai and

Trenbath, 1993). Consequently, spatial arrangement of 2:2 pattern could be influenced assimilates accumulation of the plant by the effects on IAA activity where IAA starts the movement of carbohydrates during their manufacture (Awan *et al.*, 1999). Moreover, light has a profound effect on the growth and development of plants (Briggs and Olney, 2001). Accordingly, photosynthetic pigments such as carotenoids contents, chlorophyll a and b increased with changing concentrations of IAA (Parvaiz, 2014).

These findings imply that plant growth and development involved biochemical reactions that are sensitive to solar radiation. Growing two soybean ridges alternating with two maize ridges increased ear leaf N that appeared to be associated with single leaf photosynthetic rates. So, a higher quantity of IAA would be required to the photosynthetic process in the presence of high light intensity, indicating that there was an increase in the efficiency of photosynthesis process in maize plant of 2:2 pattern than that of sole culture.

Clearly, advantage of 2:2: pattern could be due to this pattern formed better above – ground conditions especially light intensity for maize growth and development than those of sole culture (Metwally *et al.*, 2009).

With regard to mixed pattern, maize plant of this pattern had higher ear leaf N content than that of sole culture. This pattern increased ($P \leq 0.05$) ear leaf N content by 6.64 percent compared to sole culture. It is observed that growing soybean with maize on the same ridge promoted rhizobia growth in rhizosphere of maize root compared to those in rhizosphere of sole maize (Figure2). Consequently, this spatial arrangement pattern could be increased nodulation of

soybean roots through formation of more nodules during growth and development of soybean plant. Many important aspects of plant – soil interactions such as plant nutrient acquisition (Uren and Reisenauer, 1988) and root colonization by rhizosphere microorganisms (Baker, 1991) are mediated by rhizosphere processes. It is noticed that

spatial arrangement of mixed pattern increased inter-specific competition between the intercrops for basic growth resources and led to N stress for soybean where efficiency of maize as the C_4 crop for N and water use was higher than soybean as C_3 crop (Ghannoum *et al.*, 2011).

Figure 2. Effect of cropping systems, maize plant distributions, mineral N fertilizer and their interactions on ear leaf N and IAA contents, rhizobia total count in rhizosphere of maize roots at 75 days from maize sowing.

These results reveal that vegetative growth and development of maize plant benefited from the available fixed N by soybean (Figure 1) which reflected positively on the ear leaf N content (Figure 2). Similar results were obtained by Abaidoo and Kessel (1989) who found that the increase in nodulation might be due to the formation of more nodules through more secondary infections in legumes as a response to N stress generated by the maize utilization of the available soil N, legume efficiency at utilizing soil N, as well as, its potential in biological N_2 – fixation.

Also, mixed pattern decreased ($P \leq 0.05$) ear leaf IAA content of maize plant by 4.27 percent compared to that of sole culture. Obviously, large quantities of ear leaf IAA

content could be exhausted for increasing the ability of plant potential to delay ear leaf senescence which maintaining photosynthetic integrity during grain filling period (Figure 2). So, it may be possible that the increase in ear leaf N content with reduction in concentration of IAA ear leaf indicating soybean can maintain better distribution of available fixed N which might be increased the strength of physiological source of maize plant by increasing chlorophyll (Wareing *et al.*, 1968) and effective age of leaves (Nooden and Leopold, 1978). Hence, soybean is a key strategy for improving productivity of maize plant and maintaining fertility of the soil under intercropping condition. Optimal amount of phosphorus (P) and potassium

(K) in the soil cannot be utilized efficiently if N is deficient in plants where N mediates the utilization of P, K and other elements in plants (Brady, 1984). Accordingly, it seems that soybean could be controlled the size of sink and source of maize plant of mixed pattern. These findings imply that advantage of mixed pattern due to this pattern formed better under – ground conditions for maize growth and development than those of sole maize. These results are in accordance with those obtained by Odongo *et al.* (1988) who concluded that inoculation of soybean seeds increased N concentration and accumulation in maize grains. Also, Ribaut and Pilet (1994) reported that increased levels of IAA have been correlated with reduced growth. Moreover, Taiz and Zeiger (2002) showed that one of the effective factors that can control the size of sink and source are plant growth regulators. However, Alves *et al.*, (2011) found that inoculation with the strain BR11417 of *Herbaspirillum seropedicae* contributed in average with 26 % of the N necessary for the development of maize. Furthermore, Hayat *et al.* (2012) revealed one the mechanisms of the plant growth promoting bacteria – mediated enhancement of crop growth is production of hormones, *i.e.* IAA.

Maize plant distributions

Maize plant distributions affected significantly light intensity at middle and bottom of plant (Table 2). Increasing number of maize plants from one to two plants/hill by increasing plant spacing from 30 to 60 cm (with the same plant density/unit area) increased ($P \leq 0.05$) light intensity at middle and bottom of the plant by 3.41 and 6.70 percent, respectively. Accordingly, it is expected that one plant/hill had not enough space to grow and competition for solar radiation compared to two plants/hill.

On the other hand, ear leaf N and free IAA contents were affected significantly by maize plant distributions (Figure 2). Increasing number of plants from one to two plants/hill increased ($P \leq 0.05$) ear leaf N content by 3.04 percent, but it decreased ($P \leq 0.05$) free IAA content of ear leaf by 2.63 percent at 75 days from maize sowing. These data may be due to growing two plants/hill at wide plant spacing decreased intra-specific competition between plants for basic growth resources especially solar radiation where the stimulation of IAA–aspartic acid synthesis by light caused depletion of free IAA (Zelena, 2000). IAA plays a key role in both root and shoot development (Reinhardt *et al.*, 2003). Consequently, light quantity, quality, direction and duration have great impact on photosynthesis and other growth and developmental events in plants (Franklin and Whitelam, 2005). These findings indicate that growth and development of different parts of the plant may be acclimated by increasing plant spacing from 30 to 60 cm which improved the translocation of water and nutrients between root and leaves of the plant especially maize is one of the C₄ plants and is immune for light saturation. Obviously, the plant had high ability for acclimation by increasing number of plants/hill with good utilization of nutrients and water (Abdel-Galil *et al.*, 2014). These results are in accordance with those observed by Sharwood *et al.* (2014) who

concluded that leaf N concentration tended to be lower in the shaded plants relative to the control because of shade can perturb CO₂ concentration mechanisms efficiency as evidenced by changed leakiness during photosynthesis process. They added that long-term acclimation to low light in maize causes a reduction in bundle – sheath cell leakiness.

Mineral N fertilizer

Light intensity at middle and bottom of plant was affected by mineral N fertilizer (Table 2). Light intensity at middle and bottom of plant was decreased significantly by increasing mineral N fertilizer rate from 4 to 6 g N/plant without any significant differences between the medium (5 g N/plant) and the highest (6 g N/plant) mineral N fertilizer rates. Increasing mineral N fertilizer rate from 4 to 5 g N/plant decreased ($P \leq 0.05$) light intensity at middle and bottom of plant by 2.45 and 4.13 percent, respectively, at 75 days from maize sowing. Also, increasing mineral N fertilizer rate from 4 to 6 g N/plant decreased ($P \leq 0.05$) light intensity at middle and bottom of plant by 2.50 and 4.13 percent, respectively, at 75 days from maize sowing. These data indicate that intercepted solar radiation by the plant canopy was affected positively by increasing mineral N fertilizer rate from 4 to 5 or 6 g N/plant that increased ability of the plant for convert more solar energy to chemical energy.

On the other hand, ear leaf N and IAA contents were affected significantly by mineral N fertilizer (Figure 2). Ear leaf N content was increased significantly by increasing mineral N fertilizer rate from 4 to 6 g N/plant, meanwhile the reverse was true for ear leaf IAA, without any significant differences between the medium (5 g N/plant) and the highest (6 g N/plant) mineral N fertilizer rates. Increasing mineral N fertilizer rate from 4 to 5 or 6 g N/plant increased ($P \leq 0.05$) ear leaf N content by 9.60 or 11.38 percent, respectively, at 75 days from maize sowing. Also, increasing mineral N fertilizer rate from 4 to 5 or 6 g N/plant decreased ($P \leq 0.05$) ear leaf IAA content by 8.75 or 9.79 percent, respectively, at 75 days from maize sowing. These results may be due to adding 5 or 6 g N to the plant decreased the root / shoot ratio that improved N uptake by decreasing IAA synthesized in tissues of the plant. IAA is a molecule that is synthesized by plants and a few microbes (Basse *et al.*, 1996). These results are in agreement with those obtained by Baldotto *et al.* (2012) who found that with increasing rates of fertilizers, there was a decrease in the root / shoot ratio, confirming the increased availability of nutrients in the root environment. Also, Pavlíková *et al.* (2012) showed that N is a macronutrient present in many key biological molecules and it is available for plants predominantly as NO₃⁻ and NH₄⁺ that reduced IAA concentration where there is a correlation between NO₃⁻ and IAA ($R^2 = 0.77 - 0.94$).

Response of cropping systems to maize plant distributions

Light intensity at middle and bottom of plant, ear leaf N and free IAA contents were affected significantly by the interaction between cropping systems and maize plant distributions (Table 2 and Figure 2). Growing two maize

plants/hill in 2:2 pattern had the highest light intensity at middle and bottom of plant and ear leaf N content but it had the lowest free IAA content of ear leaf compared to those of the other treatments. It is observed that soybean played a major role in enhancing maize productivity of 2:2 pattern by decreasing plant density to 50 percent of sole culture at wide plant spacing. Accordingly, this interaction increased light penetration within canopies of both species compared to those of the other treatments (Metwally *et al.*, 2009).

However, mixed pattern integrated with wide plant spacing to reach the similar or less response of 2:2 pattern for light interception, ear leaf N and free IAA contents compared to sole culture. Growing one maize plant/hill spaced at 30 cm in mixed pattern could be decreased the export of carbohydrates from the shoot to the root of both species and decreased the ratio of root biomass to shoot biomass (Thomley, 1972) that could be affected negatively translocation of assimilates. Dry matter production often shows a positive correlation with the amount of intercepted radiation by crops in intercropping system (Sivakumar and Virmani, 1980). Growing two maize plants/hill spaced at 60 cm improved efficiency of soybean photosynthesis process that promoted rhizobia growth in rhizosphere of maize root (100 percent of sole maize density) at 75 days from maize sowing (Figure 2); especially the morphological and physiological differences between non-legumes and legumes benefit their mutual association (Akuda, 2001). These data indicate that each of these two factors act dependently on light intensity at middle and bottom of plant, ear leaf N and free IAA contents meaning that cropping systems responded differently ($P \leq 0.05$) to maize plant distributions for light intensity at middle and bottom of plant, ear leaf N and free IAA contents.

Response of cropping systems to mineral N fertilizer

Light intensity at middle and bottom of plant, ear leaf N and free IAA contents were affected significantly by the interaction between cropping systems and mineral N fertilizer (Table 2 and Figure 2). Maize plant of 2:2 pattern that received 6 g N had the highest light intensity at middle and bottom of plant and ear leaf N content but it had the lowest free IAA content of ear leaf compared to that of the other treatments. These data may be attributed to plant density of 2:2 pattern (50 percent of sole culture) contributed positively in increasing light transmission within intercrops and enhanced efficiency of photosynthesis process. Consequently, this interaction could be increased synthesis of amino acids into vegetative tissues of the plant.

On the other hand, mixed pattern integrated with mineral N fertilizer to reach the similar or less response of 2:2 pattern to mineral N fertilizer for light intensity at middle and bottom of plant, ear leaf N and free IAA contents. These data may be due to plant density of mixed pattern (100 percent of sole culture) contributed negatively in efficiency of light transmission within intercrops and affected negatively photosynthesis process. The reduction in photosynthesis process could be reflected on the reduction in amino acids into vegetative tissues of the plant. It is

important to mention that maize plant of mixed pattern that received 5 g N had the same contents of ear leaf N and free IAA when received 6 g N. Clearly, adding 5 g N to the plant enhanced nodulation of adjacent soybean root in rhizosphere of maize root (Figure 1) and promoted rhizobia growth in rhizosphere of maize root (Figure 2). The greater competition of maize for available soil N at high bean populations, when legumes were nearer to maize rows, might have stimulated root nodulation of intercropped beans (Abaidoo and Kessel, 1989). Moreover, symbiotic N fixation begins after the formation of nodules then reaches maximum activity in the early stages of pod filling, and subsides in the late reproductive stage (George and Singleton, 1992). These findings imply that there was adequate period of maize vegetative growth for inter-specific competition with soybean on fixed N to benefit it. By adding 5 g N to the plant, it is observed that soybean fixed about 20 percent of maize N requirements where nodulation and N fixation by legumes is adversely affected by higher rates of fertilizer N (Dogra and Dudeja, 1993). These data reveal that each of these two factors act dependently on light intensity at middle and bottom of plant, ear leaf N and free IAA contents meaning that cropping systems responded differently ($P \leq 0.05$) to mineral N fertilizer for light intensity at middle and bottom of plant, ear leaf N and free IAA contents.

Response of maize plant distributions to mineral N fertilizer

Light intensity at middle and bottom of plant, ear leaf N and free IAA contents were affected significantly by the interaction between maize plant distributions and mineral N fertilizer (Table 2 and Figure 2). Growing two plants/hill that received 12 g N had the highest light intensity at middle and bottom of plant and ear leaf N content but it had the lowest free IAA content of ear leaf compared to the other treatments. These data may be due to growing two plants/hill at wide plant spacing increased light transmission within intercrops and led to a positive effect on efficiency of photosynthesis process. These data show that each of these two factors act dependently on light intensity at middle and bottom of plant, ear leaf N and free IAA contents meaning that maize plant distributions responded differently ($P \leq 0.05$) to mineral N fertilizer for light intensity at middle and bottom of plant, ear leaf N and free IAA contents.

Response of cropping systems and mineral N fertilizer to maize plant distributions

Light intensity at middle and bottom of plant, ear leaf N and free IAA contents were affected significantly by the interactions among cropping systems, maize plant distributions and mineral N fertilizer (Table 2 and Figure 2). Growing two maize plants/hill in 2:2 pattern that received 12 g N had the highest light intensity at middle and bottom of plant and ear leaf N content but it had the lowest free IAA content of ear leaf which formed the best bioengineered treatment compared to the other treatments. These data reveal that there was effect ($P \leq 0.05$) of cropping systems x maize plant distributions x mineral N fertilizer on light

intensity at middle and bottom of plant, ear leaf N and free IAA contents.

Yield and its attributes

Cropping systems

Ear leaf area, number of ears/plant, ear weight, 100 – kernel weight, grain yield/plant, grain, seed and total yields/ha were affected significantly by cropping systems (Tables 3 and 4). Intercropping soybean with maize increased significantly ear leaf area, number of ears/plant, ear weight and grain yield/plant compared to sole culture, meanwhile the reverse was true for 100–kernel weight, grain and total yields/ha. As a result of intercropping; ear leaf area, ear weight and grain yield/plant were increased ($P \leq 0.05$) by 4.93, 7.04 and 3.76 %, respectively, compared to sole culture. These results may be attributed to there was yield advantage occurred because growth resources such as light, water, and nutrients are more completely absorbed and converted to crop biomass by the intercrop over time and space as a result of differences in competitive ability for growth resources between the component crops (Tsubo *et al.*, 2001).

Maize plant of 2:2 pattern had the highest ear leaf area, number of ears, ear weight and grain yield/plant than that of the other patterns, meanwhile, the opposite trend was true for 100 – kernel weight, grain and total yields/ha. These results may be attributed to maize of 2:2 pattern benefited greatly from environmental climatic resources and decreased intra-specific competition for basic growth resources that reflected on the highest grain yield/plant compared to that of the other patterns. Clearly, the advantage of alternating ridges of this pattern could be due to soybean gave more space for adjacent maize plant to grow well by decreasing plant density to 50 percent of sole culture and increased light intensity between plants (Metwally *et al.*, 2009). However, plant density of 2:2 pattern was not sufficient to produce the highest grain and total yields/ha.

In other words, spatial arrangement of 2:2 pattern formed suitable climatic environmental conditions for growth and development which reflected positively on ear leaf area (Table 3) that contributed largely in higher photosynthates in the developing ears/plant compared to the other patterns (Table 4). Similar results were obtained by Metwally *et al.* (2009) who found that intercropping pattern (2:2) recorded higher grain yield / unit area than sole culture.

Accordingly, spatial arrangement of 2:2 pattern improved maize productivity through producing high amount of ear leaf N content that enhanced growth and development by exhausting large quantities of IAA for producing more assimilates that partitioned to the developing ears compared to the other treatments (Figure 2). Spatial arrangement has an important influence on the degree of competition between crops (Addo – Quaye *et al.*, 2011).

Although plant density was identical between mixed pattern and sole culture, however, mixed pattern increased grain and total yields/ha compared to sole culture. Growing soybean with maize on the same ridge had higher grain and

total yields/ha than sole culture. There is evidence that leguminous plants can benefit the intercrop cereals in the same season through N excretion (Eaglesham *et al.*, 1981) and nodule decomposition (Bonetti, 1991). Plant growth and dry weight was increased by plant growth promoting rhizobacteria application (Zahir *et al.*, 1998). Clearly, mixed pattern played an important role to improve edaphic environmental conditions in rhizosphere of intercropped maize roots (Wiehe and Höflich, 1995) which reflected positively on ear leaf N and free IAA contents (Figure 2) that maximized carbon assimilation and crop productivity (Dreccer *et al.*, 2000) compared to sole culture. Obviously, the fixed N by legume can be use by intercropped cereals during their growing period and this N is an important resource for the cereals (Shen and Chu, 2004). Moreover, Gao *et al.* (2010) found that the grain yield of maize as an intercrop were significantly greater than those of maize as a sole crop. Soybean facilitates N uptake of the intercropping maize (Shu – Min and Fan, 2011) and consequently, N symbiotic fixation has contributed to facilitate N uptake for intercropped maize (Latati *et al.*, 2013).

Maize plant distributions

Ear leaf area, ear weight, 100 – kernel weight, grain yield / plant, grain, seed and total yields/ha were affected significantly by maize plant distributions, meanwhile, number of ears/plant was not affected (Tables 3 and 4). Increasing number of plants from one to two plants/hill by increasing plant spacing from 30 to 60 cm (with the same plant density/unit area) increased significantly ear leaf area, ear weight, grain yield/plant, grain and total yields/ha. In other words, doubling number of plants from one to two plants/hill increased ($P \leq 0.05$) grain yield/plant, grain and total yields/ha by 1.21, 2.00 and 3.11 %, respectively. Although symmetry of plant density was found between two types plant distribution, however, wide plant spacing had higher ear leaf N content than the other one (Figure 2). Obviously, growing two plants/hill led to largely balance in plant-to-plant competition for climatic and edaphic environmental conditions that enhanced ear leaf area for convert more solar energy to chemical energy and more translocation of photosynthates metabolites to the sink (ears), especially leaf N is distributed in parallel to light distribution (Anten *et al.*, 1995). The leaves of plants grown in the shade often exhibit what is called the shade avoidance syndrome (Smith, 2000). These results are in accordance with those obtained by Kessel and Roskoski (1988) who indicated that maize grown at the 40 cm row spacing was under more N stress than maize grown at wider rows pacing. Also, Metwally *et al.* (2009) found that total leaf area, grain yields per plant and per ha were increased by increasing plant spacing from 30 to 60 cm. Moreover, Yin *et al.* (2011) showed that the estimated bundle – sheath conductance values on leaf – area basis ranged from 1.4 to 10.3 mmol m⁻²s⁻¹ and were affected more by leaf N content than by leaf age, although bundle–sheath conductance tended to decrease as leaves became older. Under the condition of ambient CO₂ and saturating irradiance, photo respiratory loss accounted for

3–5 % of fixed carbon for the high N, versus 1–2 % for the low–N, leaves. Finally, Jiang *et al.* (2013) concluded that the reductive activity of maize roots in all soil layers and dry

weights of shallow roots were decreased significantly under narrow plant spacing conditions, resulting in lower root biomass and yield reduction at maturity.

Table 3. Effect of cropping systems, maize plant distributions, mineral N fertilizer and their interactions on ear leaf area, number of ears per plant, ear weight and one hundred kernel weight, combined data cross 2012 and 2013 seasons.

Cropping system	Maize plant distributions	Ear leaf area (cm ²)				Number of ears per plant			
		4 g N /plant	5 g N /plant	6 g N /plant	Mean	4 g N /plant	5 g N /plant	6 g N /plant	Mean
2:2 pattern	1P/30cm	916.00	929.33	932.33	925.88	1.066	1.080	1.086	1.077
	2P/60cm	919.33	932.33	936.33	929.33	1.070	1.086	1.090	1.082
Mean		917.66	930.83	934.33	927.60	1.068	1.083	1.088	1.079
Mixed pattern	1P/30cm	841.00	865.00	864.66	856.88	1.006	1.020	1.023	1.016
	2P/60cm	846.00	867.66	867.00	860.22	1.010	1.016	1.026	1.017
Mean		843.50	866.33	865.83	858.55	1.008	1.018	1.024	1.016
Average of intercropping		880.58	898.58	900.08	893.08	1.038	1.050	1.056	1.048
Sole culture	1P/30cm	839.00	853.33	857.00	849.77	1.003	1.023	1.023	1.016
	2P/60cm	844.33	854.00	859.00	852.44	1.006	1.026	1.030	1.020
Mean		841.66	853.66	858.00	851.10	1.004	1.024	1.026	1.018
Average of maize plant distributions		865.33	882.55	884.66	877.51	1.025	1.041	1.044	1.036
Average of N fertilizer rates		869.88	884.66	887.44	880.66	1.028	1.042	1.048	1.039
L.S.D.0.05 Cropping system (C)		867.60	883.60	886.05	879.08	1.026	1.041	1.046	1.037
L.S.D.0.05 Maize plant distributions (D)					3.86				0.06
L.S.D.0.05 Mineral N fertilizer (N)					3.01				N.S.
L.S.D.0.05 C x D					2.92				N.S.
L.S.D.0.05 C x N					4.08				N.S.
L.S.D.0.05 N x D					4.15				N.S.
L.S.D.0.05 C x D x N					3.11				N.S.
					4.51				N.S.

Table 3. Continued.

Cropping system	Maize plant distributions	Ear weight (g)				100 – kernel weight (g)			
		4 g N /plant	5 g N /plant	6 g N /plant	Mean	4 g N /plant	5 g N /plant	6 g N /plant	Mean
2:2 pattern	1P/30cm	200.66	203.66	208.33	204.21	34.32	33.72	32.89	33.64
	2P/60cm	204.66	207.66	212.66	208.32	33.75	33.37	32.81	33.31
Mean		202.66	205.66	210.49	206.26	34.03	33.54	32.85	33.47
Mixed pattern	1P/30cm	181.00	189.33	190.33	186.88	34.89	33.72	34.04	34.21
	2P/60cm	184.66	192.33	193.33	190.10	34.35	32.99	33.52	33.62
Mean		182.83	190.83	191.83	188.49	34.62	33.35	33.78	33.91
Average of intercropping		192.74	198.24	201.16	197.37	34.32	33.44	33.31	33.69
Sole culture	1P/30cm	177.66	183.33	187.33	182.77	35.25	34.98	34.53	34.92
	2P/60cm	180.66	187.00	190.33	185.99	35.07	34.50	34.28	34.61
Mean		179.16	185.16	188.83	184.38	35.16	34.74	34.40	34.76
Average of maize plant distributions		186.44	192.10	195.33	191.29	34.82	34.14	33.82	34.26
Average of N fertilizer rates		189.99	195.66	198.77	194.80	34.39	33.62	33.53	33.84
L.S.D.0.05 Cropping system (C)		188.21	193.88	197.05	193.04	34.60	33.88	33.67	34.05
L.S.D.0.05 Maize plant distributions (D)					4.02				0.39
L.S.D.0.05Mineral N fertilizer (N)					3.39				0.26
L.S.D.0.05 C x D					3.22				0.22
L.S.D.0.05 C x N					4.08				0.40
L.S.D.0.05 N x D					4.17				0.42
L.S.D.0.05 C x D x N					3.61				0.33
					4.33				0.49

Table 4. Effect of cropping systems, maize plant distributions, mineral N fertilizer and their interactions on maize grain yields per plant and per ha, soybean seed and total yields per ha, combined data across 2012 and 2013 seasons.

Cropping system	Maize plant distributions	Grain yield per plant (g)				Grain yield per ha (ton)			
		4 g N /plant	5 g N /plant	6 g N /plant	Mean	4 g N /plant	5 g N /plant	6 g N /plant	Mean
2:2 pattern	1P/30cm	142.30	145.12	146.49	144.63	4.11	4.20	4.27	4.19
	2P/60cm	145.26	146.36	147.75	146.45	4.30	4.40	4.50	4.40
Mean		143.78	145.74	147.12	145.54	4.20	4.30	4.38	4.29
Mixed pattern	1P/30cm	137.80	140.14	138.72	138.88	6.94	7.08	7.07	7.03
	2P/60cm	139.14	141.21	139.94	140.09	7.01	7.11	7.12	7.08
Mean		138.47	140.67	139.33	139.49	6.97	7.09	7.09	7.05
Average of intercropping		141.12	143.20	143.22	142.51	5.58	5.69	5.73	5.66
Sole culture	1P/30cm	135.28	136.51	137.10	136.29	6.56	6.75	6.86	6.72
	2P/60cm	137.09	138.83	139.29	138.40	6.73	6.84	6.98	6.85
Mean		136.18	137.67	138.19	137.34	6.64	6.79	6.92	6.78
Average of maize plant distributions	1P/30cm	138.46	140.59	140.77	139.94	5.87	6.01	6.06	5.98
	2P/60cm	140.49	142.13	142.32	141.64	6.01	6.11	6.20	6.10
Average of N fertilizer rates		139.47	141.36	141.54	140.79	5.94	6.06	6.13	6.04
L.S.D.0.05 Cropping system (C)					1.11				0.16
L.S.D.0.05 Maize plant distributions (D)					0.87				0.12
L.S.D.0.05 Mineral N fertilizer (N)					0.51				0.10
L.S.D.0.05 C x D					1.12				0.21
L.S.D.0.05 C x N					1.26				0.27
L.S.D.0.05 N x D					0.98				0.14
L.S.D.0.05 C x D x N					1.35				0.33

Table 4. Continued.

Cropping system	Maize Plant distributions	Soybean seed yield per ha (ton)				Total yield per ha (ton)			
		4 g N /plant	5 g N /plant	6 g N /plant	Mean	4 g N /plant	5 g N /plant	6 g N /plant	Mean
2:2 pattern	1P/30cm	1.62	1.57	1.66	1.61	5.73	5.77	5.93	5.81
	2P/60cm	1.76	1.71	1.73	1.73	6.06	6.11	6.23	6.13
Mean		1.69	1.64	1.69	1.67	5.89	5.94	6.08	5.97
Mixed pattern	1P/30cm	1.66	1.61	1.68	1.65	8.60	8.69	8.75	8.68
	2P/60cm	1.84	1.88	1.80	1.84	8.85	8.99	8.92	8.92
Mean		1.75	1.74	1.74	1.74	8.72	8.84	8.83	8.80
Average of intercropping		1.72	1.69	1.71	1.70	7.30	7.39	7.45	7.38
Sole culture	1P/30cm	3.03	3.03	3.03	3.03	6.56	6.75	6.86	6.72
	2P/60cm	3.03	3.03	3.03	3.03	6.73	6.84	6.98	6.85
Mean		3.03	3.03	3.03	3.03	6.64	6.79	6.92	6.78
Average of maize plant distributions	1P/30cm	1.64	1.59	1.67	1.63	6.96	7.07	7.18	7.07
	2P/60cm	1.80	1.79	1.76	1.78	7.21	7.31	7.37	7.29
Average of N fertilizer rates		1.72	1.69	1.71	1.70	7.08	7.19	7.27	7.18
L.S.D.0.05 Cropping system (C)					0.12				0.24
L.S.D.0.05 Maize plant distributions (D)					0.05				0.21
L.S.D.0.05 Mineral N fertilizer (N)					N.S.				0.11
L.S.D.0.05 C x D					0.13				0.24
L.S.D.0.05 C x N					N.S.				0.26
L.S.D.0.05 N x D					N.S.				0.32
L.S.D.0.05 C x D x N					N.S.				0.38

Mineral N fertilizer

Ear leaf area, ear weight, 100-kernel weight, grain yield/plant, grain and total yields/ha were affected significantly by mineral N fertilizer, meanwhile, number of ears/plant and seed yield/ha were not affected (Tables 3 and 4). Increasing mineral N fertilizer rates from 4 to 6 g N/plant increased ($P \leq 0.05$) ear leaf area, ear weight, grain yield/plant,

grain and total yields/ha without any significant differences between the highest (6 g N/plant) and the medium (5 g N/plant) mineral N fertilizer rates for these traits. These results could be due to adding 5 or 6 g N to the plant produced the highest ear leaf N content and the lowest free IAA content of ear leaf (Figure 2) that contributed greatly in photosynthesis process at 75 days from maize sowing (Table 2). It is observed that

growth regulators increase the strength of physiological source by increasing chlorophyll (Wareing *et al.*, 1968) and effective age of leaves (Nooden and Leopold, 1978) which finally led to increase in grain yield/plant. Some researchers have shown that spraying IAA on leaves considerably increased the growth rate of maize (Koter *et al.*, 1983).

These results are in the same context with those obtained by Bojović and Marković (2009) who found that N content influenced from presence and ratio mineral elements in the soil and it was close link with chlorophyll content. Also, Hokmalipour and Darbandi (2011) revealed that chlorophyll was increased significantly by increasing N fertilizer levels.

Response of cropping systems to maize plant distributions

Ear leaf area, ear weight, 100-kernel weight, grain yield/plant and grain, seed and total yields/ha were affected significantly by the interaction between cropping systems and maize plant distributions, meanwhile, number of ears/plant was not affected (Tables 3 and 4). Growing two maize plants/hill spaced at 60 cm in 2:2 pattern had the highest ear leaf area, ear weight and grain yield/plant compared to those of the other treatments. These data may be due to plant density of 2:2 pattern integrated with wide plant spacing to increase light transmission within maize canopy (Table 2) which improved the efficiency of photosynthesis process. This improvement had a great ecological importance in crop productivity that reflected on ear weight and grain yield/plant.

On the other hand, maize plants of mixed pattern that grown at wide plant spacing had the highest grain and total yields/ha compared to those of the other treatments. Clearly, growing two maize plants/hill spaced at 60 cm increased light transmission within maize + soybean canopies under mixed pattern. Accordingly, this positive interaction improved efficiency of photosynthesis process in soybean plants that resulted in promoting rhizobia growth in rhizosphere of maize root (Figure 2) by BNF. Accordingly, growing two maize plants/hill spaced at 60 cm under mixed pattern enhanced growth and development of maize plant by absorbed more N through fixation of atmospheric N and exhausted large quantities of IAA for producing more vegetative organs compared to the other treatments (Figure 2). It is important to mention that soybean played a major role to form better under-ground conditions for maize growth and development. BNF of the legume is not always reduced, but is dependent on the legume's ability to intercept light (Fujita and Oforu - Budu, 1996). These data reveal that each of these two factors act dependently on these traits except number of ears/plant meaning that cropping systems responded differently ($P \leq 0.05$) to maize plant distributions for ear leaf area, ear weight, 100-kernel weight, grain yield/plant, grain, seed and total yields/ha. Similar results were obtained by Metwally *et al.* (2009) who indicated that number of ears/plant was not affected by the interaction between cropping systems and maize plant distributions.

Response of cropping systems to mineral N fertilizer

Ear leaf area, ear weight, 100-kernel weight, grain yield/plant, grain and total yields/ha were affected significantly by the interaction between cropping systems and

mineral N fertilizer, meanwhile, number of ears/plant and seed yield/ha were not affected (Tables 3 and 4). Maize plant of 2:2 pattern that received 6 g N had the highest ear leaf area, ear weight, grain yield/plant compared to that of the other treatments. Although there was high intra-specific competition between the same species for mineral N fertilizer under sole culture which reflected negatively on ear leaf area, ear weight, 100-kernel weight and grain yield/plant, however, plant density of sole culture that received 6 g N/plant played a major role in grain yield/ha. Fertilizer N applied in excess of crop needs may result when soil inorganic N content is not adequately considered (Keeney, 1987). Maize plant of mixed pattern that received 4 g N did not achieve the highest grain and total yields/ha because N requirement of legumes can be met by both mineral N assimilation and symbiotic N_2 – fixation (George and Singleton, 1992). Also, the quantity of biologically fixed N can be reduced if soil available N is far below 10 kg/ha (Van Kessel and Hartley, 2000).

Also, maize plant of mixed pattern that received 5 g N had the highest grain and total yields/ha compared to that received 4 or 5 N. These data may be due to adding 5 g N to the plant promoted rhizobia growth in rhizosphere of maize roots and reflected positively on N uptake and IAA content in tissues of the plant (Figure 2) which contributed strongly in enhancing efficiency of photosynthesis process (Table 2). Clearly, mixed pattern interacted with 5 g N/plant to form suitable edaphic environmental conditions by decreasing inter-specific competition between both species for N use through N_2 fixation to produce the highest grain and total yields/ha compared to the other treatments (Table 4). Intercropping conditions of cereals and legumes would be valuable because the component crops can utilize different sources of N (Chu *et al.*, 2004). These results are in accordance with those obtained by Latati *et al.* (2013) who revealed that common bean intercropped with maize decreased inter-specific competition for N use through N_2 fixation, especially at low N concentration.

Moreover, maize plant of mixed pattern that received 6 g N did not achieve the highest grain and total yields/ha. Adding 6 g N to the plant affected negatively rhizobia activity in rhizosphere of maize roots and efficiency of biological N fixation by adjacent soybean plants (Figure 2). Although, nitrate (NO_3^-) is easily absorbed by plants at high rates, however, nitrate inhibits nitrogenase activity in legume plant nodules (Streeter, 1988). Adding commercial N fertilizer to legumes, whether straight seeded or inabland, the bacteria that actually fix the N can become lazy and N fixing declines (Braunwart, 2001). These data show that each of these two factors act dependently on these traits except number of ears/plant and seed yield/ha meaning that cropping systems responded differently ($P \leq 0.05$) to mineral N fertilizer for ear leaf area, ear weight, 100-kernel weight, grain yield/plant, grain and total yields/ha.

Response of maize plant distributions to mineral N fertilizer

Ear leaf area, ear weight, 100-kernel weight, grain yield/plant, grain and total yields/ha were affected significantly by the interaction between maize plant

distributions and mineral N fertilizer, meanwhile, number of ears/plant and seed yield/ha were not affected (Tables 3 and 4). Growing two plants/hill spaced at 60 cm that received 12 g N gave the highest ear leaf area, ear weight, grain yield/plant, grain and total yields/ha compared to the other treatments. The insignificant effect between maize plant distributions and mineral N fertilizer on number of ears/plant and seed yield/ha shows that maize plant distributions responded similarly ($P > 0.05$) to mineral N fertilizer for number of ears/plant and seed yield/ha.

Response of cropping systems and maize plant distributions to mineral N fertilizer

Ear leaf area, ear weight, 100-kernel weight, grain yield/plant, grain and total yields/ha were affected significantly by the interaction of cropping systems x maize plant distributions x mineral N fertilizer, meanwhile, number of ears/plant and seed yield/ha were not affected (Tables 3 and 4). Growing two maize plants/hill in 2:2 pattern that received 12 g N gave the highest ear leaf area, ear weight and grain yield/plant compared to that of the other treatments formed the best bioengineered treatment. The highest grain and total yields/ha was obtained by intercropping soybean on both sides of maize ridge with growing two maize plants/hill that received 10 g N formed the best bioengineered treatment. The insignificant effect of cropping systems x maize plant distributions x mineral N fertilizer for number of ears/plant and seed yield/ha shows that cropping systems and maize plant distributions responded similarly ($P > 0.05$) to mineral N fertilizer for number of ears/plant and seed yield/ha.

N use efficiency (NUE)

Cropping systems

Partial factor productivity (NUE-PFP) expressed as crop yield per unit of N applied (Roberts, 2008) are indicative of the degree of economic and environmental efficiency in use of nutrient inputs. In this study, NUE ranges from 44.85 to 149.36 kg grain yield per kg nutrient applied (Figure 3). NUE values of 40–80 kg/kg are usual with values > 60 kg/kg for NUE being common in well-managed systems or at low levels of N use, or at low soil N supply (Doberman, 2007). Maize plant of 2:2 pattern had the lowest ($P \leq 0.05$) NUE compared to that of the other patterns. These results may be due to plant density of 2:2 pattern reached 50 percent of sole culture which affected negatively grain and total yields/ha (Table 4), meanwhile maize plant of mixed pattern had the highest NUE compared to that of sole culture due to plant density reached 100 percent of sole culture. Obviously, NUE may be influenced by the complex interplay between many biological processes under mixed pattern compared to the others. These results indicate that promotion of rhizobia growth in rhizosphere of maize roots by soybean (Figure 2) led to an increase in absorption fixed N uptake from soil by maize roots. Consequently, this status led to more assimilation into amino acids that serve as N carriers throughout the plant and N transport from source to sink tissues throughout plant development, especially during the grain filling period (Table 4). Similar results were obtained by Gao *et al.* (2010) who showed that N uptake of maize as an intercrop was

significantly greater than those of maize as a sole crop. Also, Zhang *et al.* (2015) indicated that intercropping systems reduced use of N fertilizer per unit land area and increased relative biomass of intercropped maize, due to promoted photosynthetic efficiency of border rows and N utilization during symbiotic period.

Maize plant distributions

Increasing number of plants from one to two plants/hill by increasing plant spacing from 30 to 60 cm increased ($P \leq 0.05$) NUE by 1.91 percent in the combined data across 2012 and 2013 seasons (Figure 3). The results of our study show that growing maize plants at wide spacing could be decreased intra-specific competition for basic growth resources, especially solar radiation which increased ear leaf area and resulted in a positive effect on dry matter accumulation compared to the other one. Obviously, doubling plant number from one to two plants/hill led to largely balance in plant-to-plant competition for climatic and edaphic environmental conditions that enhanced ear leaf area for convert more solar energy to chemical energy and more translocation of photosynthates metabolites to the sink (ears), especially leaf N is distributed in parallel to light distribution (Anten *et al.*, 1995) and consequently increase in grain and total yields/ha (Table 4).

Mineral N fertilizer

NUE was affected significantly by mineral N fertilizer (Figure 3). Adding 5 g N to the plant increased ($P \leq 0.05$) NUE by 97.74 percent compared to those that received 6 g N/plant. These results reveal that NUE was influenced by available soil N in the experimental soil and it was close link with mineral N fertilizer. It is observed that NUE values emerging from this study apply to low levels of N use, or at low soil N supply and contributed positively in increase of photosynthesis process efficiency and consequently grain yield/plant (Table 4).

Response of cropping systems to maize plant distributions

Growing two maize plants/hill in mixed pattern had the highest NUE compared to those of the other treatments (Figure 3). It is noticed that growing soybean with two maize plants/hill at wide spacing on the same ridge increased light transmission within canopies of both species and in turn promoted rhizobia growth in rhizosphere of maize roots which facilitated more N uptake in tissues of the plant compared to that of the other treatments (Table 2 and Figure 2).

Growing soybean on both sides of maize ridge led to the highest grain yield/plant, grain and total yields/ha due to a better N-use efficiency of C_4 compared to C_3 plants, since the rate of photosynthesis per unit N in the leaf is increased (Oaks, 1994). These data indicate that each of these two factors act dependently on NUE meaning that cropping systems responded differently ($P \leq 0.05$) to maize plant distributions for NUE.

Response of cropping systems to mineral N fertilizer

Maize plant of mixed pattern that received 5 g N had the highest NUE compared to that of the other patterns (Figure 3). These data may be due to adding 5 g N to the plant promoted rhizobia growth in rhizosphere of maize roots and reflected positively on N uptake and IAA content in tissues of the plant

(Figure 2) which contributed strongly in enhancing efficiency of photosynthesis process and the grain yield /plant compared to the other treatments (Table 4). This result expected

considering each cropping system has different number of plants and each will have their own N uptake.

Figure 3. NUE as affected by cropping systems, maize plant distributions, mineral N fertilizer and their interactions, combined data across 2012 and 2013 seasons.

Response of maize plant distributions to mineral N fertilizer

Growing two plants/hill spaced at 60 cm that received 10 g N had the highest NUE compared to that of the other treatments (Figure 3). These results indicate that growing two maize plants/hill interacted with 10 g N to decrease inter-specific competition between both species for N use through N_2 fixation and produced the highest grain and total yields/ha compared to the other treatments (Table 4). These data show that each of these two factors act dependently on NUE meaning that maize plant distributions responded differently ($P \leq 0.05$) to mineral N fertilizer for NUE.

Response of cropping systems and maize plant distributions to mineral N fertilizer

Growing two maize plants/hill in mixed pattern that received 10 g N had the highest NUE compared to those of the other treatments (Figure 3) and formed the best bioengineered treatment. These data reveal that there was effect ($P \leq 0.05$) of cropping systems x maize plant distributions x mineral N fertilizer on NUE.

4. Conclusion

Our results indicate that bioengineering has a great importance for maximizing intercrop yields. The best bioengineered treatment was varied according to the intercropping pattern. Soybean increased productivity of maize plant through modifying the light environment around maize plants that directly affected positively ear leaf free IAA and N contents in 2:2 pattern. Spatial arrangement of mixed pattern influenced strongly maize productivity of unit area though maintaining the recommended plant density of both species that integrated strongly with positive effects of soybean plant on soil N availability. Soybean could be a biological tool to compensate the one-third of the amount of

mineral N fertilizer for maize requirements.

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