American-Eurasian J. Agric. & Environ. Sci., 14 (1): 11-16, 2014
ISSN 1818-6769

DOI: 10.5829/idosi.aejaes.2014.14.01.11893

Growth Promotion in Maize with Diazotrophic Bacteria in Succession With Ryegrass and White Clover

Leandro Hahn, Enilson Luiz Saccol de Sá, Rafael Goulart Machado, William Rosa da Silva, Suelen Oldra, Raquel Garibaldi Damasceno and André Schönhofen

Departament of Soil, Agronomy Faculty,
Federal Universityof Rio Grande do Sul (UFRGS),
90001-970, Porto Alegre, RS, Brasil

Abstract: Winter cover plants and diastrophic bacteria inoculation are indicated as alternatives for nitrogen supply (N) and increase in maizegrowth. The objective of the present study was to study associated rhizobia and diastrophic bacteria in maize growth promotion in succession to crops of annual ryegrass *(Lolium multiflorum)* and white clover *(Trifolium repens)*. The experiment was carried out on the UFRGS Agronomic Experimental Stationin the 2010/2011 and 2011/2012 growing seasons. White clover and ryegrass were cropped in the winter and maize in succession to these crops. The plants were inoculated with the UFRGS Vp16 and SEMIA 222 rhizobia and a mix of three *Azospirillum* isolates, UFRGS LG-R, UFRGS EL-S and UFRGS MS, intwo N doses, 70 and 140 kg ha^{-1}. Inoculation with UFRGS Vp16rhizobium and the mixture of three *Azospirillum* isolates increased growth of the maize plants cropped in succession to ryegrass and white clover.

Key words: *Zea mays* · Biological nitrogen fixing · 16S DNAr · *Azospirillum* · *Burkholderia*

INTRODUCTION

Nitrogen (N) is the nutrient that has the greatest effect on yield increase in maize crops, but less than 50% N applied by fertilizer is used by the plants[1]. The result of this low efficiency is soil and underground water contamination that harms health and endangers agricultural sustainability [2].

Instead of increasing themaize yield by using higher doses of mineral nitrogen, cover plants can be used during the winter as an alternative to supply N to maize cropped in succession. These crops improve the physical and chemical properties of the soil, especially when leguminous plants are cropped in these areas [3].

A second alternative to supply N to the maize crop is by biological fixing by associative bacteria[4] or increase in its absorption by the plant [5]. Several growth promoting bacteria can significantly increase growth and grain yield of various cereals when these are used as inoculants [6]. Growth promotion by these bacteria can be a consequence of biological nitrogen fixing [4] plant regulator production [7], antagonism against plant pathogens by siderophore production[8] or increased phosphorus availability [9].

The capacity of maize to establish plant growth promoting rhizospheric and/or endophytic relationships with several bacteria genera is well documented [10]. Lately there has been great interest in studying and using rhizobia isolated from legume nodules as growth promoters in many grasses. Positive results using this technique have already been obtained in rice[11] and maize plants [12, 13].

The object of the present study was to assess efficiency of inoculating symbiotic rhizobia in legumes and associative diazotrophic bacteria in maize growth promotion in succession to winter crops such as ryegrass and white clover.

MATERIALS AND METHODS

Experimental details and treatments: The experiment was carried out in the field in an area at the Agronomic

Corresponding Author: Leandro Hahn, Departament of Soil, Agronomy Faculty, Federal University of Rio Grande do Sul (UFRGS), 90001-970, Porto Alegre, RS, Brasil.

Experimental Station of the Federal University of Rio Grande do Sul, located in the municipality of Eldorado do Sul, RS, Brazil (latitude 30°39'S, longitude 51°06'W and altitude 46 m). The soil is classified as typical dystrophic Ultisol and presented at implantation the following characteristics (0-20 cm depth): 5.6 pH H_2O, 24 g kg^{-1} OM, 12.5 mg dm^{-3} P, 76 mg dm^{-3} K, 2.6 $cmol_c$ dm^{-3} Ca, 1.1 $cmol_c$ dm^{-3} Mg, 0.5 $cmol_c$ dm^{-3} Al.

The experiment was set up in 2010 and conducted for two years, in the 2010/2011 and 2011/2012 growing seasons.In the winter white clover (*Trifolium repens*) and annual ryegrass (*Lolium multiflorum*) were cropped. Maize was cultivated between the 2010 winter crop and after the 2011 winter crop during the summer. The plants were inoculated with the following rhizobia: UFRGS Vp16, belonging to the Rhizobia Culture Collection at UFRGS, that was isolated from white clover nodules and selected because of its high nitrogen fixing efficiency [14]; SEMIA 222, a strain released for inoculant production for *Trifolium repens*; and the mixture of three *Azospirillum* sp. isolates,UFRGS El-S UFRGS Lg1-R and UFRGS M-S, belonging to the Rhizobia Culture Collection at UFRGS that were selected because of their high nitrogen fixing efficiency in maize cropped in Rio Grande do Sul and *in vitro* indoleacetic acid (IAA) production [15].

Two N doses were tested in addition to inoculating the bacterial isolates: 140 kg ha^{-1} that corresponded to the complete nitrogen dose for maize and half the dose (70 kg ha^{-1}N).

The maize hybrid Pioneer 30F53 was sown in December in the two growing seasons after desiccating thewinter crops with 1.5 L glyphosate. In the plots with white clover, 1.5 L glyphosate was re-applied 15 days after the first application. Maize was sown by hand in the no tillage system. Five rows of maize were sown with 0.6 m between row spacing, for a final population of 65,000 plants ha^{-1}. In the treatments with 140 kg ha^{-1}, in addition to the 15 kg ha^{-1} N applied via formulated fertilizer in the sowing, 15 kg ha^{-1} was applied on the soil surface after sowing and 110 kg ha^{-1}N in cover when the plants were at the V_{4-5} stage[16]. In the treatments with 70 kg ha^{-1}, 55 kg ha^{-1}N was applied on soil surface when the plants were at the V_{4-5} stage.

In the two years of experimenting weeds were controlled after emergence with herbicide application when the maize plants were at the V_3 stage. Spray irrigation was applied in water shortage periods. A randomized block design was used with four replications and the plots were 7 m long and 3 m wide.

Bacterial Inoculant Preparation: To produce the bacterial inoculant, the UFRGS Vp16 and SEMIA 222 rhizobia were inoculated in liquid YM medium[17] and the *Azospirillum* isolates inoculated in Dygs liquid medium[18] placed in an orbital incubator at 28°C and shaken at 120 rpm for six days. The bacterial broths were mixed in sterilized water and applied by hand with a watering can over the row shortly after sowing. For the UFRGS Vp16 and SEMIA 222 treatments,5 mL broth were mixed with $8x10^8$ cells mL^{-1} in 8 L water and $3.3x10^8$ cells were applied per m^{-2}. For the treatments that received the mixture of the three *Azospirillum* isolates, 5 mL broth with $7.5x10^8$ cells mL^{-1} of each isolate were mixed in 8 L water and $9x10^8$ cells were applied $perm^2$. The cell concentration was counted in a Neubauer chamber.

Assessments: The relative leaf chlorophyll contents were assessed by reading the values of the Falker Chlorophyll Index (FCI) at the V_{6-7} stage in 10 plants randomly chosen and identified from the three central rows of the plot, excluding 0.5 m from the ends. These values were determined on the last fully opened leaf blade. Three readings were taken on the middle third of each leaf blade to obtain a mean value. At the R_6 stage the 10 plants identified previously were cut and after drying in a chamber at 65°C were weighed and ground to determine the canopy dry matter. The grain yield was obtained by manual harvest of the ears of the plants on the three central rows of the plots, excluding 0.5 m from the ends and corrected to 130 g kg^{-1} moisture. The nitrogen content was determined from the collected grains according to [19].

The Relative Efficiency Index (REI) was also determined in the canopy dry matter production and the grain yield of maize in succession to winter crops. For this the equation below was used, were *TRAT1* corresponds to the value of the variable of the treatments inoculated with addition of N equivalent 100% of the complete N doses, $CONT_{N/2}$corresponds to the value of the variable of the control treatments with the addition of N equivalent to 50% of the complete N dose and $CONT_N$ corresponds to the value of the variable of the control treatments with addition of N equivalent to 100% of the complete N doses.

$$REI(\%) = \left(\frac{\left(TRAT_t - CONT_{N/2} \right) - \left(CONT_N - CONT_{N/2} \right)}{CONT_N - CONT_{N/2}} \right) \times 100$$

Taxonomic Identification of the Isolates: To identify the isolates, the total DNA of the bacteria was extracted using the Wizard kit (Promega) and the 16S DNArgene was amplified using the F515 and R806 universal primers. The PCR reactions were made in a 20 µl volume containing 1 µL DNA, 1x PCR buffer, 2 mM $MgCl_2$, 200 µM of eachdNTP, 0.2 µM of the primers and 1U Platinum Taq DNA polymerase (Invitrogen). The cycles used for amplification were: an initial denaturationcycle at 94°C for 2 min, 25 cycles including denaturation for 45 seconds at 94°C, annealing for 45 seconds at 55°C and extension for 1 min at 72°C followed by a final 6 min extension phase at 72°C. The fragments were sequenced using the ABI-PRISM 3100 Genetic Analyzer equipment (Applied Biosystems). The sequences of the DNAr region of the isolates were researched in the GenBank using the BLAST 2.0 program.

Statistical Analysis: The results were submitted to analysis of variance using the ASSYSTAT statistical program [20] and the means compared by the Tukey test ($P<0.05$).

RESULTS

Field Experiments: Regarding the control treatment with 70 kg ha^{-1}N, inoculating the three *Azospirillum* isolates promoted growth in the maize plants. This was observed in the 2011/2012 growing season in the increase of the CDM yield in the succession to ryegrass using 70 kg ha^{-1}N and succession to clover with 140 kg ha^{-1}N (Table 1) and in the Relative Efficiency Index (REI) values (Table 2). Inoculating the *Azospirillum* isolates presented the highest REI in the CDM (64.0%) that was obtained in the 2011/2012 growing season in succession to clover.

Inoculation with the UFRGS Vp16 rhizobium, compared to the treatment control, also promoted growth in the maize plants. This was observed in the increase in CDM production in the succession to white clover in the 2011/2012 growing season with 140 kg ha^{-1}N and the maize plant grain yield in succession to ryegrass with 70 kg ha^{-1}N in the 2010/2011 growing season that was equivalent to a grain increase of 13.9% or 1.1 Mg ha^{-1}. Higher values were also observed with the bacteria

Table 1: Canopy dry matter (CDM) production, Falker chlorophyll index(FCI), grain nitrogen content and grain yield of maize inoculated with diazotrophic bacteria in the 2010/2011 and 2011/202 growing seasons with 70 and 140 kg ha^{-1} nitrogen

Nitrogen (kg ha^{-1})		70				140			
		CDM (Mg ha^{-1})							
Growing season	Previous crop	Control	*Azospirillum*	SEMIA 222	UFRGS Vp16	Control	*Azospirillum*	SEMIA 222	UFRGS Vp16
2010/2011	Ryegrass	18.8 Aa	20.9 Aa	19.1 Ba	20.6 Aa	24.3 Aa	25.2 Aa	25.5 Aa	24.0 Aa
	White clover	16.9 Ba	17.9 Ba	16.9 Ca	17.9 Ba	18.3 Ca	18.6 Ba	18.8 Ba	19.0 Ba
2011/2012	Ryegrass	17.3 Bb	19.2 Aa	17.9 Aab	18.9 Bab	22.3 Aa	22.1 Ba	23.4 Aa	23.0 Aa
	White clover	19.2 Aa	20.2 Aa	19.9 Aa	21.9 Aa	22.3 Ab	24.3 Aa	22.4 Aab	24.0 Aa
CV = 8,4%									
		FCI in V_{5-6}							
2010/2011	Ryegrass	45.0 ABa	48.2 Aa	46.0 Aa	48.0 ABa	49.9 Aa	50.4 Aa	51.0 Aa	49.2 Aa
	White clover	41.6 Ba	43.9 Ba	40.2 Ba	45.1 Ba	42.9 Ba	44.5 Ba	43.3 Ba	44.6 Ba
2011/2012	Ryegrass	44.7 Aa	44.3 Ba	47.2 Aa	46.5 Ba	50.1 Aa	50.9 Aa	49.6 ABa	51.2 Ba
	White clover	47.7 Aa	48.3 Aa	48.2 Aa	51.0 Aa	55.1 Aa	53.2 Aa	53.2 Aa	54.2 ABa
CV = 4,6%									
		Grain nitrogen content (g kg^{-1})							
2010/2011	Ryegrass	13.8 Aa	13.3 Aa	13.9 Aa	13.6 Aa	14.8 Aa	14.0 Aa	14.0 Aa	14.5 ABa
	White clover	11.6 Ba	11.4 Ba	11.8 Ba	11.3 Ba	11.0 Ba	11.4 Ba	11.0 Ba	11.8 Ba
2011/2012	Ryegrass	12.8 Aa	13.6 Aa	12.4 Aa	14.5 Aa	15.5 Aa	12.5 Aa	14.8 Aa	15.5 Aa
	White clover	13.1 Aa	14.1 Aa	13.1 Aa	14.3 Aa	14.8 Aa	15.3 Aa	14.5 Aa	15.0 Aa
CV = 8,4%									
		Grain yield (Mg kg^{-1})							
2010/2011	Ryegrass	7.9 Ab	8.5 Aab	8.2 Aab	9.0 Aa	9.9 Aa	9.5 Aa	9.4 Aa	9.9 Aa
	White clover	6.2 Ba	6.3 Ba	6.3 Ba	6.1 Ba	7.2 Ba	7.1 Ba	7.3 Ba	7.1 Ba
2011/2012	Ryegrass	7.6 Ba	7.6 Ba	8.0 Ba	8.5 Ba	9.5 Ba	9.7 Aa	9.6 Ba	9.9 Ba
	White clover	9.4 Aa	10.0 Aa	9.7 Aa	9.9 Aa	11.3 Aa	11.1 Aa	11.2 Aa	11.5 Aa
CV = 12,1%									

Treatment means followed by the same lowercase letter on the line, in each nitrogen dose and uppercase letter in the column for each growing season, did not differ (Tukey ($P<0.05$) CV = coefficient of variation

Table 2: Relative efficiency index (REI) (%) of inoculation of the treatments with diazotrophic bacteria on the canopy dry matter growth (CDM) and grain yield of maize cropped in succession to ryegrass and white clover in the2010/2011 and 2011/202 growing seasons with 70 and 140 kg ha^{-1} nitrogen

		2010/2011		2011/2012	
Previous crop	Treatment	CDM	Grain yield	CDM	Grain Yield
Ryegrass	*Azospirillum*	15.7	-18.6	15.9	11.7
	SEMIA 222	22.3	-24.1	22.3	4.6
	UFRGS Vp16	-5.6	3.1	13.8	19.3
White clover	*Azospirillum*	21.6	-6.9	64.0	-10.8
	SEMIA 222	33.1	10.7	-28.1	-2.7
	UFRGS Vp16	44.5	-5.6	55.1	12.3

Positive REI values of the inoculated treatment refer to the increase in the value of the variable in relation to the control treatments with half of the N dose (70 kg ha^{-1} N) and the complete N dose (140 kg ha^{-1} N). Negative values refer to lower values of the variable compared to the control treatments

inoculation in this treatment (Table 2) as observed in the 2011/2012 growing season for grain yield of the plants cropped in succession to ryegrass (19.3%) and CDM production of the plants cropped in succession to white clover (44.5%).

Taxonomic Identification of the Isolates: The genetic sequencing of the 16S rDNA of the isolates used in the present study identified the rhizobium UFRGS Vp16 as belonging to *Burkholderia* sp. with 100% similarity with the accession JN975051.1. The diazotrophic bacteria were identified as belonging to *Azospirillumbrasilense*, with 99% similarity of the UFRGS El-S isolate to accession GU256438.1, 100% similarity to the UFRGS Lg1-R isolate with accession FN813475.1 and 99% similarity to the UFRGS M-S isolate with accession JF700491.1.

DISCUSSION

Inoculating bacteria of the *Azospirillum* genus to promote growth in maize has shown positive results in several studies [21, 5, 22]. The three *Azospirillum* cultures used in the present experiment were isolated by [15] from maize plants collected in the state of Rio Grande do Sul, Brazil. These isolates performed best among 30 plants for indoleacetic acid production (IAA), a plant regulator from the group of auxines and for N fixing ability. In the present study, growth promotion in maize plants was probably due to different growth promotion mechanisms, such as plant regulator production [7], increased nutrient absorption [5], N fixing [4] or increased phosphorus availability [9].

It is pointed out that in the 2010/2011 growing season, with 70 kg ha^{-1}N and in succession to the two winter crops, inoculating with the *Azospirillum* isolates stimulated increase in grain yield compared to the control treatment without inoculation that was equivalent to a 14% increase or 1.100kg ha^{-1} in grain. However, as in the inoculation treatment with the *Azospirillum* isolates that are capable of biological nitrogen fixing (BNF) in grasses, differences were not observed regarding the treatments inoculated with the SEMIA 222 and UFRGS Vp16 rhizobia, that are not nitrogen fixers in non-leguminous plants, that may infer that these results were due to the existence of other growth promotion mechanisms and not to BNF. A greater nutrient absorption capacity of the soil is suggested [5], nitric oxide production [23] and 1-aminocyclopropane-1-carboxylatedesaminase (ACC desaminase) activity [24] and especially plant hormone production such as auxines [7].

There are few studies on inoculating maize crops with bacteria of the *Burkholderia* genus to which the UFRGS Vp16 rhizobium belongs [25, 26]. The UFRGS Vp16 rhizobium was isolated from a white clover nodule and selected because of its high nitrogen fixing efficiency [13]. Inoculating symbiont rhizobia in leguminous plants has been carried out in several cereals especially rice [11], but there are few studies on inoculation in maize [12, 13].

Maize cropped in succession to white clover in the 2010/2011 growing system presented lower values for the variables tested compared to maize cultivated in succession to ryegrass (Table 1). The smaller maize plant growth was probably due to competition for water, light and nutrients in the maize plantvegetative period with the white clover plants remaining from the winter that did not desiccate prior to sowing the maize. The maize growth period that extends from stage V_0 (emergence) to V_7 (seven opened leaves) is considered the most critical for competition with other plants [27]. Unlike the 2010/2011 growing season, in the 2011/2012 growing season there were higher values for the variables in the maize cropped in succession to clover compared to the maize cropped in succession to ryegrass succession. In the 2011/2012 growing season the clover plants were totally controlled before the maize was sown and there was no competition with the maize plants as had happened in the 2010/2011 growing season. The greater growth of the maize plants obtained in succession to white clover in the 2011/2012 growing season can be explained by the N supply from the clover plants [28-31].

CONCLUSION

The results of the present study indicated that there is great potential to increase maize production by inoculating diazotrophic bacteria efficient in growth promotion together with the use of winter cover plants that could result in lower production costs and less potential environmental impact from nitrogen fertilizers.

It can be concluded that inoculating the UFRGS Vp16 rhizobium and the mixture of the three *Azospirillum* isolates, UFRGS LG-R, UFRGS EL-S and UFRGS MS increased growth in maize plants cropped in succession to ryegrass and white clover.

REFERENCES

1. Halvorson, A.D., G.A. Peterson and C.A. Reuele, 2002. Tillage system and crop rotation effects on dry land crop yields and soil carbon in the Central Great Plains. Agron. J., 94: 1429-1436.
2. Tilman, D., 1998.The greening of the green revolution.Nature, 396: 211-221.
3. Diekow, J., J. Mielniczuk, H. Knicker, C. Bayer, D.P. Dick and I. Kogel□Knabner, 2005. Soil C and N stocks as affected by cropping systems and nitrogen fertilization in a southern Brazil Acrisol managed under no□tillage for 17 years. Soil Till Res., 81: 87-95.
4. Montañez, A., C. Abreu, P.R. Gill, G. Hardarson and M. Sicardi, 2009. Biological nitrogen fixation in maize (Zea mays L.) by ^{15}N isotope-dilution and identification of associated culturable diazotrophs. Biol. Fert Soils, 45: 253-263.
5. Hungria, M., R.J. Campo, E.M.S. Souza and F.O. Pedrosa, 2010.Inoculation with selected strains of Azospirillum *brasilense* and *A. lipoferum improves* yields of maize and wheat in Brazil. Plant Soil, 331: 413-425.
6. Kennedy, I.R., A.T.M.A. Chouhury and M.L. Kecskés, 2004. Non-symbiotic bacterial diazotrophs in crop-farming systems; can their potential for plant growth promotion be latter exploited? Soil Biol. Biochem. 36: 229-1244.
7. Dobbelaere, S., J. Vanderleyden and Y. Okon, 2003. Plant growth-promoting effects of diazotrophs in the rhizosphere.Crit Rev Plant Sci., 22: 107-149.
8. Scher, F.M. and R. Baker, 1982. Effect of *Pseudomonas putida* and a synthetic iron chelator on induction of soil suppressiveness to *Fusarium* wilt pathogens. Phytopathology, 72: 1567-1573.
9. Sessitsch, A., J.G. Howieson, X. Perret, H. Antoun and E. Martínez Romero, 2002. Advances in Rhizobium research. Crc Rev Plant Sci., 21: 323-378.
10. Caballero-Mellado, J.L. Martinez-Aguilar, V.Y. PAREDES and P. Estrada de los Santos, 2004. Burkholderiaunamae sp. nov.and N_2 fixing rizospheric and endophytic species. Int. J. Syst. Evol., 54: 1165-1172.
11. Bhattacharjee, R.B., P. J ourand, C. Chaintreuil, B. Dreyfus, A. Singh and S.N. Mukhopadhyay, 2012.Indole acetic acid and ACC deaminase-producing Rhizobium leguminosarumbv. trifolii SN10 promote rice growth and in the process undergo colonization and chemotaxis. BiolFert Soils, 48: 173-182.
12. Gutierrez-Zamora, M.L. and E.M. Romero, 2001. Natural endophytic association between Rhizobium etli and maize (Zea mays L.). J. Biotechnol., 91: 117-126.
13. Bécquer, C.J., U.B. Salas, U. Ávila, L. Palmero, J.A. Nápoles, L. Ulloa, Y. Suárez and O.L. Colina, 2011. Selection of rhizobium strains, inoculated in maize (Zea mays, L.), in field conditions in cattle ecosystems of Sancti Spiritus, Cuba. Cuban J. Agr. Sci., 45:4.
14. Alves, J.B., E.L.S Sá and A.L.Muniz, 2012. Selection of rhizobia for *Trifolium repens* in flooded soil conditions. Biotemas, 25: 39-45.
15. Röesch, L.F.W., P.D.Q. Quadros, F.A.O. Camargo and E.W. Triplett, 2007. Screening of diazotrophic bacteria Azopirillum spp. for nitrogen fixation and auxin production in multiple field sites in southern Brazil.World J. Microb. Biot., 23: 1377-1383.
16. Ritchie, S., J. Hanway and G. Benson, 1992. How a Maize Plant Develops. Available at: http://www.extension.iastate.edu/hancock/info/maize.htm (Acessed: 12 August 2013)
17. Vincent, J.M., 1970. Manual for the practical study of root nodule bacteria.Oxford: Blackwell Scientific, pp: 164.
18. Rodrigues Neto, J., V.A. Malavolta Júnior and O. Victor, 1986. Meio simples para o isolamento e cultivo de *Xanthomonas campestres* pv. citri. tipo B. Summa Phytopathol., 12: 16 .
19. Tedesco, M.J., C. Gianello, C.A. Bissani, H. Bohnen and S.J. Volkweiss, 1995. Analysis of soil, plants and other materials. 2. ed. Departamento de Solos da UFRGS, Porto Alegre, Brazil, pp: 174.

20. Silva, F.A.S.E. and C.A.V. Azevedo, 2009. Principal Components Analysis in the Software Assistat-Statistical Attendance. In: WORLD CONGRESS ON COMPUTERS IN AGRICULTURE, 7, Reno: American Society of Agricultural and Biological Engineers.
21. Jacoud, C., D. Job, P. Wadoux and R. Bally, 1999. Initiation of root growth stimulation by Azospirillum lipoferum CRT1 during maize seed germination. Can J. Microbiol., 45: 339-342.
22. Joe, M.M., B.Karthikeyan, P.S. Chauhan, C. Shagol, M.R. Islam, M. Deiveekasundarama and T. SA, 2012. Survival of Azospirillum brasilense flocculated cells in alginate and its inoculation effect on growth and yield of maize under water deficit conditions. Eur. J. Soil Biol., 50: 198-206.
23. Creus, C.M., M. Graziano, E.M. Casanovas, M.A. Pereyra, M. Simontacchi, S. Puntarulo, C.A. Barassi and L. Lamattina, 2005. Nitric oxide is involved in the Azospirillum brasilense-induced lateral root formation in tomato. Planta, 221: 297-303.
24. Prigent-combaret, C., D. Blaha, J.F. Pothier, L. Vial, M.A. Poirier, F. Wisniewski-Dyé, Y. and Y. Moënne-loccoz, 2008. Physical organization and phylogenetic analysis of acdR as leucine-responsive regulator of the 1-aminocyclopropane-1-carboxylate deaminase gene acdS in phytobeneficialAzospirillum lipoferum 4B and other Proteobacteria.FEMS Microbiol. Ecol., 65: 202-219.
25. Riggs, P.J., M.K. Chelius, A.L. Iniguez, S.M. Kaeppler and E.W. Triplett, 2001. Enhanced maize productivity by inoculation with diazotrophic bacteria. Aust J. Plant Physiol., 28: 829-836.
26. Miyauchi, M.Y.H., D.S. Lima, M.A. Nogueira, G.M. Lovato, L.S. Murate, M.F. Cruz, J.M. Ferreira, W. Zangaro and G. Andrade, 2008. Interactions between diazotrophic bacteria and mycorrhizal fungus in maize genotypes. Sci. Agr., 65: 525-531.
27. Kozlowski, L.A., 2002. Critical period of weed interference in maize crop based on crop phenology. Planta Daninha, 20: 365-372.
28. Paynel, F., F. Lesuffleur, J. Bigot, S. Diquelou and J.B. Cliquet, 2008. A study of N^{-15} transfer between legumes and grasses. Agron Sustain Dev., 28: 281-290.
29. Panda, B.B., Kalpesh Gaur, M.L. Kori, L.K. Tyagi, R.K. Nema, C.S. Sharma and A.K. Jain, 2009. Anti-Inflammatory and Analgesic Activity of Jatropha gossypifolia in Experimental Animal Models, Global Journal of Pharmacology, 3(1): 01-05.
30. Parmar Namita, Rawat Mukesh and J. Kumar, 2012. Vijay Camellia Sinensis Green Tea. A Review Global Journal of Pharmacology, 6(2): 52-59.
31. Jagadeeswaran, M., N. Gopal, B. Jayakar and T. Sivakumar, 2012. Simultaneous Determination of Lafutadine and Domperidone in Capsule by High Performance Liquid Chromatography, Global Journal of Pharmacology, 6(2): 60-64.