INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 3, No. 4, p. 128-134, 2013

RESEARCH PAPER OPEN ACCESS

Growth response of soybean to the application of *Bradyrhizobium japonicum* and foliar methanol spraying in field conditions

Arvin Saadpanah, Asad Rokhzadi*, Khosro Mohammadi

Department of Agronomy and Plant Breeding, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran

Key words: Biofertilizer, *Bradyrhizobium japonicum*, foliar application, methanol, soybean.

doi: http://dx.doi.org/10.12692/ijb/3.4.128-134 Article published on April 22, 2013

Abstract

Abstract

This experiment was carried out to study the effects of foliar methanol spraying and seed inoculation with *Bradyrhizobium japonicum* on phenology and growth of two soybean cultivars using a split-split plot layout with randomized complete block design in three replications. Two treatments of non-application and application of biofertilizer (*B. japonicum*) were compared in main plots. Three levels of methanol including: 0 (distilled water), 15 and 30 % (v/v) were assigned in subplots and two soybean cultivars including Clark and TMS were applied in sub-subplots. Growth stages of soybean including flowering, podding and physiological maturity, biomass dry weight and chlorophyll conent of leaves were determined. Results showed that podding stage of soybean was accelerated as the result of methanol spraying. Seed inoculation with *B. japonicum* increased the plant biomass compared with control and foliar spraying by 15% methanol produced the highest rate of plant biomass in comparison with 30% methanol and control. The highest amount of leaf chlorophyll content was recorded by using of 15% methanol and inoculation of plant with *B. japonicam*. Numbers of days from sowing to different growth stages in TMS were significantly lower than those of Clark. TMS was superior than Clark in terms of biomass production and chlorophyll content. According to the results of this experiment seed inoculation with *Bradyrhizobium* and foliar spraying by moderate concentration of methanol led to significant improvement in growth traits of soybean plants, and TMS was more compatible to regional conditions as compared with Clark cultiva.

* **Corresponding Author:** Asad Rokhzadi ✉ asadrokh@yahoo.com

Introduction

Methanol is one of the simplest organic molecules and a natural product of plant metabolism which is emitted from the leaves of most plants (Fall and Benson, 1996). A considerable proportion of methanol released from plants is probably a by-product of pectin metabolism during cell wall synthesis (McDonald and Fall, 1993; Nemecek-Marshall *et al.,* 1995). The gaseous methanol emitted from the stomata of plants can be utilized by methylotrophic bacteria as carbon and energy source, moreover these microorganisms may promote the growth of their host plant through the release of various metabolites (Abanda-Nkpwatt *et al.,* 2006).

It has been reported that foliar application of methanol enhances the growth and yield of various C_3 plants (Nonomura and Benson, 1992; Fall and Benson, 1996). Positive responses of several plants including wheat seedlings, geranium plants, winter rape and oil-seed rape to methanol application in greenhouse conditions were reported by Zbiec and Karczmarczyk (1997). Effects of methanol application on *Arabidopsis*, tobacco and tomato plants were studied by Ramirez *et al.* (2006). They showed that foliar application of methanol led to an increase in fresh and dry weight of *Arabidopsis* and tobacco plants whereas no significant increase was shown in tomato plants. In another study Hernandez *et al.* (2000) declared that growth traits of sunflower including stem length, leaf area, stem dry weight and number of floret primordial were significantly increased as the result of foliar application of methanol under controlled conditions, however methanol treated sunflowers grown in field conditions did not show significant enhancement compared with control. Zbiec *et al.* (2003) showed that various crops such as tomato, bean, sugar beet, oil seed rape when treated with methanol solutions yielded 20-30% higher than the control. Results of field and pot trials on the effects of methanol on cotton and sugarcane conducted by Madhaiyan *et al.* (2006) showed that application of 30% methanol as foliar spray significantly increased plant height, plant dry weight, leaf area, boll number and boll dry weight, leading to an increase of seed cotton yield over control. Furthermore they showed that foliar application of methanol increased the growth and yield of sugarcane (a C_4 plant) despite the previous reports claiming lacked response to foliar applied methanol in C_4 plants (Nonomura and Benson, 1992; Fall and Benson, 1996). In spite of the mentioned beneficial effects of methanol on plants, there are other reports claiming the inefficiency of foliar methanol application in plants (Wutscher, 1994; McGiffen *et al.,* 1995; Rajala *et al.,* 1998; Sinclair and Cassman, 2004).

Soybean is the most widely grown legume in the world having high levels of protein and oil in seed composition and it also promotes soil fertility through modifying the soil nitrogen budget (Meghvansi *et al.,* 2008). About half of nitrogen needs of soybean can be obtained from the air when nitrogen-fixing rhizobia bacteria are present in the soil (Elmore, 1984). The specific rhizobium of soybean is known as *Bradyrhizobium japonicum*. Soybean plants in association with *B. japonicum* can fix up to 200 kg N ha^{-1} yr^{-1} reducing the need for expensive and environmentally damaging nitrogen fertilizer (Zhang *et al.,* 2003). Establishing rhizobia or inoculation in a field that has never grown soybean is needed to ensure nitrogen fixation, therefore it is important to inoculate on any soil if soybeans are to be grown for the first time (Thelen and Schulz, 2011). Soybean has not been previously grown in the region of current experiment and there is need to inoculate soybean in order to achieve optimum N_2 fixation. In present study we examined the effects of soybean seeds inoculation with *B. japonicum* and foliar application of methanol on growth traits of two soybean cultivars under field conditions.

Materials and methods

Field conditions

This experiment was carried out at the research farm of Islamic Azad University, Sanandaj Branch (35° 10′ N, 46° 59′ E) during the spring and summer 2012.

Soil traits of the farm in the layer of 0-30 cm were included: EC 0.4 dS m^{-1}, pH 7.9, Sp 38.8%, OC 0.49%, TNV 24.9%, clay 31%, silt 39%, sand 30%, available P and K 3.03 and 232 ppm respectively.

Experimental design and plant material

The experiment was arranged in a split-split plot layout with randomized complete block design in three replications. Two treatments of non-application and application of biofertilizer (*Bradyrhizobium japonicum*) were compared in main plots. Three levels of methanol including: 0(distilled water), 15 and 30 % (v/v) were assigned in subplots and two soybean cultivars including Clark and TMS were applied in sub-subplots. Clark is an indeterminate genotype wherease TMS is determinate. Each sub-subplot included four sowing ridges 3 m in length with 50 cm space between rides and 5 cm between plants on each ridge.

Seed inoculation and sowing

Sowing and inoculation of seeds by *Bradyrhizobium japonicum* biofertilizer were done on 30 April 2012. Seeds were hand sown into open furrows on the top of ridges. In order to avoid cross infection between treatments, the seeds of uninoculation control plots were sown beforehand. Then the seeds of inoculation treatments were inoculated by *B. japonicum* inoculants suspension at the rate of 2 mL inoculants suspension per 100 g seeds. Then the inoculated seeds were dried under shed and after 15-20 minutes were hand sown. The inoculation product was obtained from Nature Biotechnology Company (nbtc), Tehran, Iran.

First irrigation was immediately performed after the sowing operation and hand hoeing was regularly done, to keep the crop free from weeds.

Methanol application

Foliar methanol treatments including 0 (distilled water), 15 and 30 % (v/v), were applied three times at 50, 65 and 80 days after sowing. The spraying of methanol was done at about 6 o'clock in the evening. In order to enhance the metabolism of methanol in the plant, glycine at the rate of 2 g per 1 L was added to all treatments of methanol and control (distilled water).

Data collection

Phenological stages of R_1 (beginning bloom), R_3 (beginning pod) and R_8 (full maturity) were determined for each experimental plot according to the method of Fehr *et al.* (1971). The stages of R_1, R_3 and R_8 were considered as 50% flowering, 50% podding and physiological maturity stages respectively. Then the number of days from sowing to the stages of 50% flowering, 50% podding and physiological maturity were recorded. At physiological maturity a 2 m^2 area of two central rows of each plot was harvested. The harvested plants were air dried and plant biomass per area unit were calculated. Content of leaf chlorophyll was estimated by SPAD-502 device (Konica-Minolta, Japan) at seed-filling stage.

Statistical analysis

The collected data were analyzed statistically by analysis of variance operations using the MSTAT-C computer package version 2.10. Means of treatments were compared by Duncan's multiple range test at the 0.05 level of significance.

Results and discussion

Crop phenology

Soybean growth stages were not affected by the main effect of biofertilizer factor (Table 1). Methanol application significantly affected the date of podding stage. In non-inoculation state, the number of days to podding was statistically decreased by foliar application of methanol compared with control (distilled water spraying) (Fig. 1). Similar result was reported by Hernandez *et al.* (2000). They showed that foliar application of methanol accelerated the final stage of floral development by 4.5 days earlier than control in sunflower.

Growth stages of flowering, podding and physiological maturity were significantly affected by cultivar factor (Table 1). Shorter growth stages were

recorded by TMS cultivar (Fig. 4). The interaction effect of biofertilizer × cultivar on number of days from sowing to 50% flowering showed that the longest duration of vegetative stage (61.4 days) was recorded by Clark with no application of biofertilizer but with the application of biofertilizer, the vegetative phase duration in Clark was significantly decreased to 56.3 days, on the other hand TMS cultivar either with biofertilizer or without biofertilizer has recorded the lowest numbers of days from sowing to flowering stage (Fig. 2). The lowest number of days from sowing to 50% podding was recorded by TMS and the longest period from sowing to podding (69.7 days) was recorded by the treatment of no application of biofertilizer in Clark cultivar (Fig. 3). TMS cultivar reached to its physiological maturity about more than 3 weeks earlier than Clark (Fig. 4). Differnces between two cultivars with regard to growth stages, may be reffered to their different growth habits. Clark is an indeterminate genotype wherease TMS is determinate, indicating that TMS may complete its growth stages more quickly than Clark.

Table 1. Analysis of variance of soybean growth traits affected by biofertilizer, methanol and cultivar factors.

Source of variation	df	Mean square				
		Days to flowering	Days to podding	Days to maturity	Plant biomass	Leaf chlorophyll
Replication	2	4.778^{ns}	2.778^{ns}	31.194^{ns}	105608.072^{ns}	1.650^{ns}
Biofertilizer (A)	1	21.778^{ns}	17.361^{ns}	4.694^{ns}	49539302.873^{**}	33.834^{ns}
E_a	2	4.111	2.778	30.528	208434.393	4.519
Methanol (B)	2	1.361^{ns}	4.861^{*}	10.111^{ns}	9686683.537^{**}	15.834^{**}
A×B	2	26.694^{ns}	9.028^{**}	8.778^{ns}	340628.199^{ns}	21.268^{**}
E_b	8	6.111	0.694	2.486	608603.286	1.351
Cultivar (C)	1	821.778^{**}	250.694^{**}	5064.694^{**}	2688014.820^{*}	234.600^{**}
A×C	1	113.778^{**}	17.361^{**}	0.694^{ns}	37268.306^{ns}	2.834^{ns}
B×C	2	4.694^{ns}	4.861^{ns}	4.111^{ns}	248679.442^{ns}	3.738^{ns}
A×B×C	2	4.694^{ns}	9.028^{*}	12.111^{ns}	40472.141^{ns}	1.364^{ns}
E_c	12	4.556	1.389	2.889	470252.327	2.021
CV (%)		3.94	1.80	1.81	10.43	3.25

ns, * and **: Non significant and significant at 5 and 1% levels of probability, respectively.

Table 2. Means comparison of plant biomass affected by by biofertilizer, methanol and cultivar factors.

Treatments	Plant biomas (kg ha^{-1})
Biofertilizer	
Uninoculation (Control)	5398.8 b
Inoculation	7745.0 a
Methanol	
0%	5762.3 b
15%	7538.5 a
30%	6415.0 b
Cultivar	
Clark	6298.6 b
TMS	6845.2 a

Mean values with same letters in a group of a column are not significantly different at P ≤ 0.05 according to Duncan's multiple range test.

Plant biomass

Analysis of data revealed that biofertilizer of *B. japonicum* and methanol application statistically influenced the plant biomass. Seed inoculation with *B. japonicum* increased the biomass up to 43.5% as compared with control (Table 2). This result was in agreement with reports by Egamberdiyeva *et al.* (2004). They declared that inoculation of soybean varieties with *B. japonicum* increased shoot dry weight up to 45% compared to uninoculated plants.

Foliar spraying of plant with 15% methanol significantly increased the plant biomass in comparison with distilled water (0% methanol) and by increasing the concentration of methanol to 30% the plant biomass started to decrease and reached to the same statistical level of control (0% methanol), even though the plant biomass was still higher than the control (Table 2). Nonomura and Benson (1992),

Zbiec and Karczmarczyk (1997) and Ramirez *et al.* (2006), similarly declared that foliar spraying of methanol increased the growth of various plants. The positive effects of methanol on growth traits of plants have been referred to the action of methanol as an inhibitor of photorespiration (Nonomura and Benson, 1992, Fall and Benson, 1996). The reduction of plant biomass as the result of spraying with 30% methanol compared with 15% methanol in our study, may be attributed to possible toxicity effects of high percentages of methanol on the plant (Nonomura and Benson, 1992).

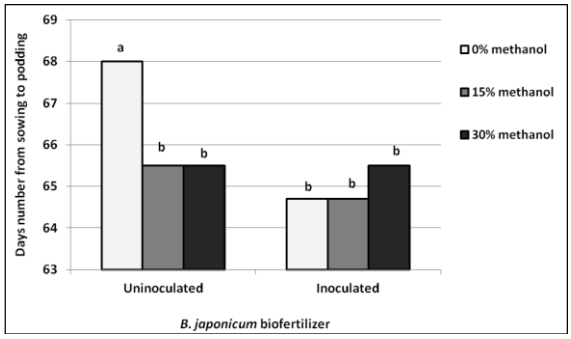

Fig. 1. Interaction effect of biofertilizer and methanol on days number from sowing to podding.

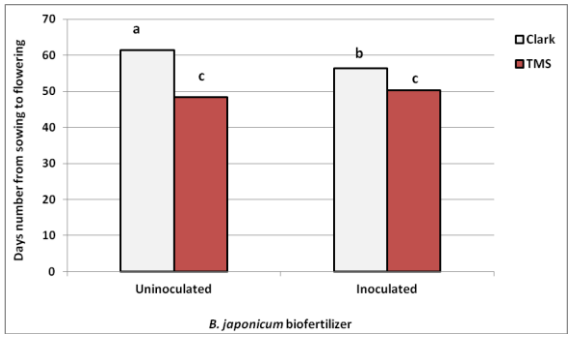

Fig. 2. Interaction effect of biofertilizer and cultivar on days number from sowing to flowering.

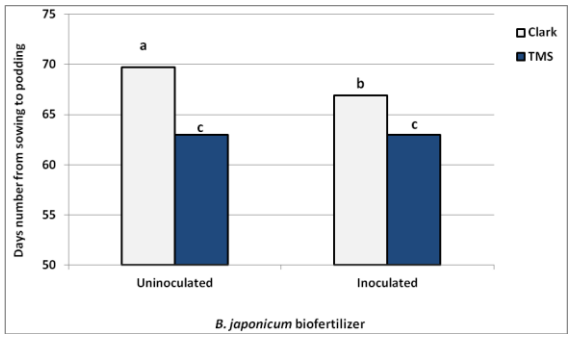

Fig. 3. Interaction effect of biofertilizer and cultivar on days number from sowing to podding.

The plant biomass produced by TMS cultivar was significantly higher than that of Clark (Table 2) which could be related to the more compatible response of TMS to regional conditions resulting in more productivity in comparison with Clark.

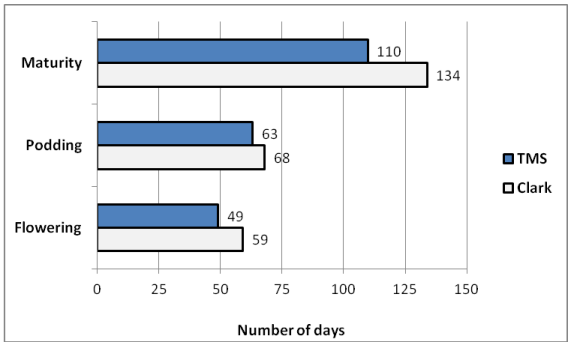

Fig. 4. Duration of growth stages in 2 soybean cultivars. Flowering: from sowing to 50% flowering, Podding: from sowing to 50% podding and Maturity: from sowing to physiological maturity.

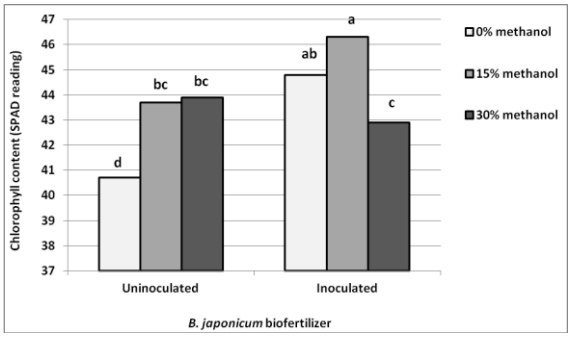

Fig. 5. Interaction effect of biofertilizer and methanol on leaf chlorophyll content (SPAD readings).

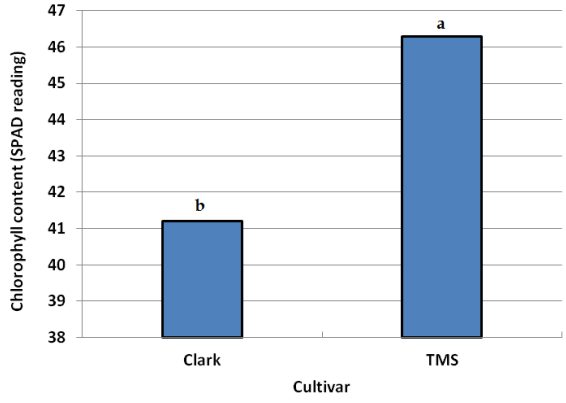

Fig. 6. Effect of cultivar on leaf chlorophyll content (SPAD readings).

Leaf chlorophyll

The content of leaf chlorophyll (based on SPAD readings) was affected by the application of

methanol (Table 1). The highest amount of chlorophyll was recorded by using of 15% methanol in the case of plant inoculation with *B. japonicum* (Fig. 5). Koukourikou-Petridou and Koukounaras (2002) in an experiment similarly reported that chlorophyll content of tomato and pepper was increased by application of methanol and glycine. In another study Paknejad *et al.* (2009) showed that moderate concentration of methanol elevated the chlorophyll content of soybean leaves, but higher concentrations of methanol decreased the content of leaf chlorophyll. On the other hand Li *et al.* (1995) previously have reported that foliar application of methanol could not change net photosynthesis and chlorophyll content of soybean leaves. Soybean cultivars in our study in terms of chlorophyll content of their leaves were significantly different. Mean content of chlorophyll recorded by TMS was higher than that of Clark (Fig. 6) suggesting that TMS is probably more efficient in exploitation of environmental factors such as carbon and nitrogen sources to produce higher contents of chlorophyll in its photosynthetic organs compared with Clark cultivar.

Conclusion

According to the results of this experiment seed inoculation with *Bradyrhizobium* and foliar spraying by methanol as a carbon source, led to significant improvement in growth of soybean plants. Considering the reduction of days number to podding as the result of methanol spraying (in the case of non-application of biofertilizer) it can be proposed that methanol may has the potential of accelerating the growth of soybean, even though further investigation will be needed in this respect. In addition to, more suitable growth traits recorded by TMS cultivar suggestes that TMS is more compatible to regional conditions as compared to Clark cultivar.

References

Abanda-Nkpwatt D, Musch M, Tschiersch J, Boettner M, Schwab W. 2006. Molecular interaction between Methylobacterium extorquens and seedlings: growth promotion, methanol consumption, and localization of the methanol emission site. Journal of Experimental Botany **57 (15),** 4025-4032.

Egamberdiyeva D, Qarshieva D, Davranov K. 2004. The Use of Bradyrhizobium to Enhance Growth and Yield of Soybean in Calcareous Soil in Uzbekistan. Journal of Plant Growth Regulation **23,** 54-57.

Elmore RW. 1984. Soybean Inoculation-When is it necessary? University of Nebraska.

Fall R, Benson AA. 1996. Leaf methanol - the simplest natural product from plants. Trends in Plant Science **1 (9),** 296-301.

Fehr WR, Caviness CE, Burmood DT, Pennington JS. 1971. Stage of development descriptions for soybeans, *Glycine max* (L.) Merrill. Crop Science **11,** 929-931.

Hernandez LF, Pellegrini CN, Malla LM. 2000. Effect of foliar application of methanol on growth and yield of sunflower (*Helianthus annuus* L.). Phyton, International Journal of Experimental Botany **66,** 1-8.

Koukourikou-Petridou M, Koukounaras A. 2002.The effect of methanol on the growth and chlorophyll content of tomato and pepper. Acta Horticulturae (ISHS)**579,** 271-274.

Li Y, Gupta G, Joshi JM, Siyumbano AK. 1995. Effect of methanol on soybean photosynthesis and chlorophyll. Journal of Plant Nutrition **18 (9),** 1875-1880.

Madhaiyan M, Poonguzhali S, Sundaram SP, Sa TM. 2006. A new insight into foliar applied methanol influencing phylloplane methylotrophic dynamics and growth promotion of cotton (*Gossypium hirsutum* L.) and sugarcane (*Saccharum officinarum* L.). Environmental and Experimental Botany **57,** 168-176.

MacDonald R, Fall R. 1993. Detection of substantial emissions of methanol from plants to the atmosphere. Atmospheric Environment **27A,** 1709-1713.

McGiffen ME, Green RL, Manthey JA, Faber BA, Downer AJ, Sakovich NJ, Aguiar J. 1995. Field tests of methanol as a crop yield enhancer. HortScience **30 (6),** 1225-1228.

Meghvansi MK, Prasad K, Harwani D, Mahna SK. 2008. Response of soybean cultivars toward inoculation with three arbuscular mycorrhizal fungi and *Bradyrhizobium japonicum* in the alluvial soil. European Journal of soil biology **44,** 316-323.

Nemecek-Marshall M, MacDonald RC, Franzen JJ, Wojciechowski CL, Fall R. 1995. Methanol emission from leaves: Enzymatic detection of gas phase methanol and relation methanol fluxes to stomatal conductance and leaf development. Plant Physiology **108,** 1359-1368.

Nonomura AR, Benson AA. 1992. The path of carbon in photosynthesis: Improved crop yields with methanol. Proceedings of the National Academy of Sciences of the USA, **89,** 9794-9798.

Paknejad F, Mirakhori M, Jami Al-Ahmadi M, Tookalo MR, Pazoki AR, Nazeri P. 2009. Physiological response of soybean (*Glycine max*) to foliar application of methanol under different soil moistures. American Journal of Agricultural and Biological Sciences **4 (4),** 311-318.

Rajala A, Karkkainen J, Peltonen J, Peltonen-Sainio P. 1998. Foliar applications of alcohols failed to enhance growth and yield of C_3 crops. Industrial Crops and Products **7,** 129-137.

Ramirez I, Dorta F, Espinoza V, Jimenez E, Mercado A, Pena-Cortes H. 2006. Effects of Foliar and root applications of methanol on the growth of arabidopsis, tobacco, and tomato plants. Journal of Plant Growth Regulation **25,** 30-44.

Sinclair TR, Cassman KG. 2004. Agronomic UFOs. Field Crops Research **88,** 9-10.

Thelen K, Schulz T. 2011. Soybean Seed Applied Inoculation. soybean management and research technology, Michigan State University

Wutscher H. 1994. The effect of methanol on orange fruit quality. HortScience **29,** 575.

Zbiec I, Karczmarczyk S. 1997. Effect of methanol on some plants. Romanian Agriculrural Research **7-8,** 45-49.

Zbiec I, Karczmarczyk S, Podsiadlo C. 2003. Response of some cultivated plants to methanol as compared to supplemental irrigation. Electronic Journal of Polish Agricultural Universities **6 (1),** #01.

Zhang H, Prithiviraj B, Charles TC, Driscoll BT, Smith DL. 2003. Low temperature tolerant Bradyrhizobium japonicum strains allowing improved nodulation and nitrogen fixation of soybean in a short season (cool spring) area. European Journal of Agronomy **19,** 205-213.