

# Effect of Urea, Humax and *Rhizobium japonicum* on yield of Soybean

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## Abstract

In order to study the effects of Urea, Humax and *Rhizobium japonicum* on yield of Soybean, a field experiment was established in 2014. It included two cultivars of soybean i.e. Habbit and L<sub>17</sub> (main factor) with six fertiliser treatments i.e. control; seed inoculated with *Rhizobium japonicum*; basal nitrogen (urea) + topdress urea at R<sub>2</sub> stage (when the flowers open in one of the two upper nodes of the main stem with a fully developed leaf visible); basal nitrogen (urea) + seed inoculated with *Rhizobium japonicum* + topdress nitrogen at R<sub>2</sub> stage; seed treated with Humax + topdress Humax at R<sub>2</sub> stage; basal nitrogen + seed treated with Humax + topdress Humax at R<sub>2</sub> stage (sub factors). A split-plot randomised complete block design with three replications was adopted. Fertiliser treatments increased seed weight and seed yield significantly. The results showed that cv. Habbit along with basal nitrogen fertiliser + urea nitrogen at R<sub>2</sub>, achieved the highest seed weight. The highest seed yield in the basal nitrogen + seed treated with Humax + topdress Humax at R<sub>2</sub> stage was observed. It can be concluded that the use of basal nitrogen in soybean is beneficial. Moreover, at the end of flowering (R<sub>2</sub>), the consumption of nitrogen can improve the potential for a higher yield.

## Keywords

Oilseed crops, nutrition, fertiliser, Humic acid, inoculation.

## Introduction

One factor that plays an important role in determining the yield potential of soybeans is the nutritional status including the supply of nitrogen (N). What is certain is that in many categories the N fixing symbiosis with bacteria does not satisfy the need. Nitrogen stress reduces the N content of the plant, thus hampering its growth. In addition, evidence does not exist to indicate a halt in the nitrogen uptake by soybeans at maturity. Biological fertilisers, including a large population of beneficial and natural micro-organisms help to preserve and maintain the soil. Fertilisers only provide the required N to plants, but organic fertilisers are claimed to also supply hormones, vitamins and amino acids. Organic fertilisers are reputed to supply the N needed for plant growth gradually through a plant's growth stages. Chemical fertilisers have been claimed to damage soil structure, while the use of organic fertilisers improves soil structure (Tanujapurhit 2008).

Osborn and Rieddel (2006) concluded that starter fertiliser (50-100 Kg urea/ha) can have positive effects on soybean in cold environments. The positive effects of N application have been demonstrated in some field studies (Taylor et al 2005; Raggio and Raggio 2007). An increase in the availability of nitrogen for soybeans during flowering, the number of nodes in plants, and the effects of the increase in the number of fertile seeds and pods increases yield (Brevendan et al. 1978). Yosefi (1994) compared the effect of N fertiliser and Nitragin on two varieties of soybean and showed that the inoculation of soybean with Nitragin (1 L/50 kg seed) increased yield (about one t/ha) in comparison to a control, but the addition of N (50 kg urea/ha) without the inoculation of soybean had little impact. Humic acid may also be effective in improving nutrient uptake via hormonal impact on plant growth and root system development (Nikbakht et al. 2008; Amiri et al. 2009). Humic acid (54 mg/L) has also been shown to stimulate the production of auxin, cytokinins and gibberellin, resulting in increased absorption efficiency and improved germination of wheat. Albarda et al. (2009) studied the effect of inoculum *Rhizobium japonicum* (125 g inoculant/50 Kg seed) on soybean grain yield and found higher yields in inoculated plots compared to non-inoculated treatments. Such results have been confirmed by a series of studies (Farahbakhsh et al. 2005; Son et al. 2006). Owing to the importance of soybean as an oilseed crop and its sensitivity to N nutrition, we decided to assess the response of two soybean cultivars to different N sources (Urea, Humax and *Rhizobium japonicum*).

## Methods

This field study was done in a split plot in a randomised complete block design with three replications during 2014 at the research farm of the Islamic Azad University of Bojnourd, Iran. The trial site was between 57 degrees, 21 minutes northern longitude and 37 degrees 29 minutes eastern latitude and 1,056 m above sea level. The treatments consisted of soybean cultivars as the main factor at two levels (including Habbit and L<sub>17</sub>) and compound fertiliser as sub-plots at six levels:

- Treatment (1), control
- Treatment (2), seed inoculated with *Rhizobium japonicum*
- Treatment (3), basal nitrogen (urea) + topdress urea at R<sub>2</sub> stage
- Treatment (4), basal nitrogen (urea) + seed inoculated with *Rhizobium japonicum* + topdress urea at R<sub>2</sub>
- Treatment (5), seed treated with Humax + topdress Humax at R<sub>2</sub>
- Treatment (6), basal nitrogen (urea) + seed treated with Humax + topdress Humax at R<sub>2</sub>.

Soil physical and chemical properties were assessed on samples collected before performing the tests on the plantation. Basal fertilisers, based on the results of soil analysis, were applied. Planting was undertaken by hand (400,000 plants / ha) on the 10 May. R<sub>2</sub> stage in soybean is when the flowers open in one of the two upper nodes of the main stem with a fully developed leaf visible (Fehr and Caviness 1980). The amount of urea used at the R<sub>2</sub> stage was 20 kg/ha and 120 kg/ha in other treatments. A commercial product Humax was applied at 1/3 L/ha with irrigation water and 1/25 litres/100 kg of seed for seed treatment. The bacteria package used contained 300 g *Bradyrhizobium japonicum* for one hectare. Each plot consisted of six 6-m-long rows. Harvesting was done when 95% of the pods were yellow and the seeds hard. To investigate the functional components of lines 3, 4 and 5 of each plot, five plants were randomly selected and transferred to the laboratory where the number of nodes on the main stem of each plant, number of pods per node, number of seeds per pod, seed weight and the number of tributaries were measured. To assess the biomass and seed yield of lines 3 and 4, 2 m<sup>2</sup> samples were taken from each plot. All data were analysed by ANOVA using SAS software and data was compared with Duncan's multiple range test.

## Results

The results of the comparison of interaction effects (Table 1) show that the lowest number of nodes on the main stem (18/47) was observed with cultivar L<sub>17</sub> and treatment 5 (Humax seed treatment + topdress Humax). In contrast, the highest number of nodes on the main stem (12/87) was observed with the Habbit cultivar and treatment 5 (Humax seed treatment + topdress Humax) ( $P < 0.05$ ). Difference between the cultivars appeared related to genetic differences. Habbit has a determinate growth habit, whereas the L<sub>17</sub> is semi- indeterminate type (Asadi 2008).

Differences in grain yield between the different soybean cultivars appeared mainly due to the number of pods per plant (Board et al. 1996). From Table 1, the highest number of pods on the main stem nodes (6/76) was observed with the Habbit cultivar and Treatment 6 (basal nitrogen + seed treated with Humax + topdress Humax). Humax appeared to increase the absorption of starter fertiliser in early growth stages due to affecting nutrient uptake and its hormonal effects. The greater number of pods per plant coincided with increased N absorption. This result is also confirmed by Brevedan et al. (1978), who reported that increasing N levels during flowering in soybean, resulted in 22% more pods per node compared to the control (no N).

The highest seed per pod was observed with cultivar L<sub>17</sub> and treatment 6 (basal nitrogen + seed treated with Humax + topdress Humax) ( $P < 0.05$ ; Table 1). It seems that N fertiliser use with Humax increased the number of seeds per pod by increasing the availability of N during pod filling. The highest 100 seed weight (17.8 g) was obtained by cv. Habbit with treatment 3 (basal nitrogen + topdress urea) which was significantly ( $P < 0.05$ ) higher than the Habbit cultivar control (13.6 g). The lower seed weight loss in the control was interpreted as N stress occurring at the beginning of pod filling when the seed or pod per plant was not significantly affected, but seed weight was significantly reduced. That stress in turn resulted in the plant producing smaller seeds (Egli et al. 1978).

**Table 1. Mean comparison of interaction effects. Treatment (1), control; Treatment (2), seed inoculated with *Rhizobium japonicum*; Treatment (3), basal nitrogen (urea) + topdress urea at R<sub>2</sub> stage; Treatment (4), basal nitrogen(urea) + seed inoculated with *Rhizobium japonicum* + topdress urea at R<sub>2</sub>; Treatment (5), seed treated with Humax + topdress Humax at R<sub>2</sub>; Treatment (6), basal nitrogen (urea) + seed treated with Humax + topdress Humax at R<sub>2</sub>. Means with similar letters not significantly different based on Duncan test (P = 0.05).**

Treatment	Node/stem	Pode/node	Seed/pod	100 seed weight (g)	Tributaries	Seed yield (kg/ha)	Biomass (kg/m <sup>2</sup> )	Harvest Index (%)	
Cultivar	Fertiliser treatments								
L <sub>17</sub>	1	16/31ab	2/93b	2/42b	14/4de	12bc	4308/3bcd	1/15bcde	0/36d
	2	13/4ab	3/5ab	2/68ab	14/88cde	14/18abc	4658/1abc	1/36abcd	0/34d
	3	15/38ab	3/95ab	2/58ab	14/48de	14/84abc	4923/3abc	1/63ab	0/36d
	4	14/8ab	4/06ab	2/56 ab	15/88abcd	15/78abc	5467a	1/73a	0/37d
	5	18/47a	4/1ab	2/78ab	17/29ab	18/29a	5023/3ab	1/4abcd	0/4abcd
	6	15/02ab	5/03ab	2/85a	15/46bcde	16/73ab	5600/1a	0/71e	0/38cd
Habbit	1	16/96ab	2/8b	2/45ab	13/56e	11/25bc	3085/2e	1/2abcde	0/43abc
	2	14/5ab	2/96b	2/43ab	17/13ab	11/1bc	3850cde	1/36abcd	0/46a
	3	14/64ab	3/8ab	2/6ab	17/82a	13/62abc	5142/1ab	1/5abc	0/39bcd
	4	14/03ab	3/48ab	2/55ab	16/8abc	10c	4062/2bcde	0/96cde	0/43abc
	5	12/87b	3/68ab	2/36b	16/33abcd	11/24bc	3467de	0/86de	0/45ab
	6	16/55ab	6/76a	2/56ab	16/46abcd	14/8abc	5767/3a	1/1bcde	0/45ab

The lowest number of branches (10) occurred in cv. Habbit and treatment 4 (basal nitrogen + seed inoculated with *Rhizobium japonicum* + topdress nitrogen) (Table 1). In contrast, the highest number of branches (18/29) was observed in cv. L<sub>17</sub> and treatment 5 (Humax + topdress Humax). This difference was significant (P = 0.05). This difference reflected the indeterminate growth habit of Habbit superiority over cv. L<sub>17</sub>. Humax fertiliser treatment is known to stimulates the production of hormones; auxin, cytokinin, and gibberellins, and increase the number of branches in the plant (Amiri et al. 2009).

Table 1 shows average yields for cv. L<sub>17</sub> of 5,467 and 5,600 kg/ha were achieved using treatments 4 and 6 respectively. The highest yield (5,767 kg per ha) was observed in cv. Habbit and treatment 6 (basal nitrogen + seed inoculated with *Rhizobium japonicum* + topdress nitrogen). The use of basal nitrogen in soybean farming appears beneficial. Moreover, at the end of flowering (R<sub>2</sub>) soil N reserves decline, and recapitalisation of resources, chemical (or biological), can improve the potential for a higher yield. The lowest yield was in the control treatment of cv. Habbit with an average of 3,085 kg/ha. There was no significant difference in yield between the 4 treatments for this cultivar (Table 1).

The highest biological yield was achieved by the L<sub>17</sub> cultivar and treatment 4 (basal nitrogen + seed inoculated with *Rhizobium japonicum* + topdress nitrogen) (Table 1). In contrast the lowest biological yield was achieved by L<sub>17</sub> cultivar and treatment 6 (Basal nitrogen + seed treated with Humax + topdress Humax). The indeterminate growth nature of L<sub>17</sub> seems to be the reason why more assimilates were produced. Azizi (1993) stated that the difference in biomass weight was not influenced by the type or level of fertiliser. The results of the comparison of interaction effects (Table 1) showed the highest harvest index related to cv. Habbit and treatments 2 and 5 (seed inoculated with rhizobium and seed treated with Humax + topdress Humax at R<sub>2</sub>). The bacteria treatment effect in cv. L<sub>17</sub> showed the lowest harvest index. It seems that cv. L<sub>17</sub> due to its semi-indeterminate growth habit produced more biomass and this led to a reduced harvest index.

## Conclusions

- The best fertiliser treatment in terms of the number of nodes on the main stem, number of pods per node, number of seeds per pod and number of tributaries was treatment 6 (basal nitrogen + seed treated with Humax + topdress Humax at R<sub>2</sub> stage).
- Highest seed yield was produced by treatment 6 (basal N + seed treated with Humax + topdress Humax at R<sub>2</sub>).
- L<sub>17</sub> and Habbit cultivars did not differ in seed yield. Habbit is recommended however for cold conditions because of its determinate growth, which prevents the coincidence of maturity phase and the cold season.
- Basic nitrogen in soybean farming is clearly beneficial.

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