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# RELATIONSHIP BETWEEN SOYBEAN VARIETIES, RHIZOBIA INOCULATION AND SPAD-502 CHLOROPHYLL METER READINGS IN WESTERN SIBERIA

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#### **Abstract**

Due to climate change and an increasing demand for food and fodder, the production of soybeans (*Glycine max*) in Russia as well as in Western Siberia increases. Within the framework of the interdisciplinary German-Russian project SASCHA a field trial was installed in Kuchak, Western Siberia (Russian Federation) in 2013 to investigate the relationship between soybean varieties, rhizobia inoculation and SPAD-502 chlorophyll meter readings. Within two German and one Siberian variety SPAD values were measured as well as numbers of nodules counted. The plants were grown in two variants (with and without inoculation) in a completely randomized block design with four replications. Only the inoculated varieties developed nodules. The German varieties showed significant higher SPAD meter readings at the latest measurement during mid of seed development. Due to low soil temperatures differences may not have occurred earlier.

Keywords: Soybean, inoculation, nodulation, chlorophyll content, nitrogen fixation

#### Introduction

Due to climate change and an increasing demand for food and fodder, the production of soybeans (*Glycine max*) in Russia as well as in Western Siberia increases (Kiselev et al., 2013; Faostat, 2011). As a legume crop species soybeans do have a close relationship between the availability of nitrogen either from N<sub>2</sub>-fixation or soil N and the net leaf photosynthetic rate (Vollmann et al., 2011). Biological nitrogen fixation requires necessarily the inoculation with specific rhizobia bacteria e.g. *Bradyrhizobium japonicum* (Werner, 1999). Soils usually lack *Bradyrhizobium japonicum* strains (Hiltbold et al., 1985). Therefore it is important to inoculate seeds especially if soy is planted on the field for the first time, otherwise no nodulation will occur (Solomon et al., 2012). High variabilities in nodulation (Cregan et al., 1989) and N<sub>2</sub> fixation (Herridge and Rose, 2000) between soybean genotypes are reported. By comparing the leaf chlorophyll content of plants with and without nodules it is possible to quantify how much inoculation may increase the N-uptake (Vollmann et al., 2011). Leaf chlorophyll metering e.g. by Minolta SPAD-502 appears to be a useful technique for determining the nitrogen status of soybeans (Gwata et al., 2004).

Within the framework of the interdisciplinary German-Russian project SASCHA (SASCHA, 2013) a field trial was installed to investigate the relationship between soybean varieties, rhizobia inoculation and SPAD-502 chlorophyll meter readings. The results were compared with a similar trial which took place in Osnabrueck, Northern Germany in 2011.

#### Materials and methods

A field trial with soybean varieties from Germany and Siberia was established at the experimental site "Kuchak" of Tyumen State University in Western Siberia (57°20'56"N, 66°3'24"E) in May 2013 (Figure 1).

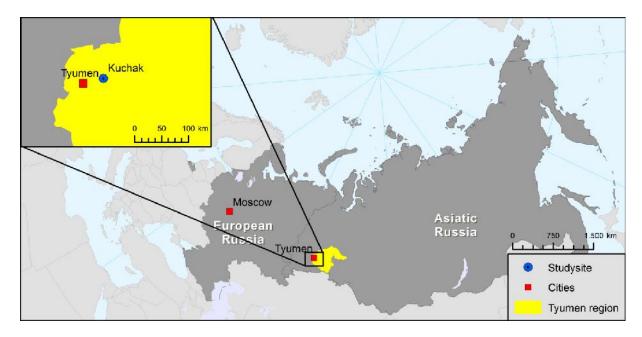


Figure 1: Experimental station "Kuchak"/ Tyumen State University in Tjumen/Western Siberia

Kuchak shows continental climate with a mean min/max temperature of -2.9/6.9° C (range from -42 to 38°C) and yearly average precipitation of 450 mm. The experimental site (podsolic soil) was irrigated during the trials as required.

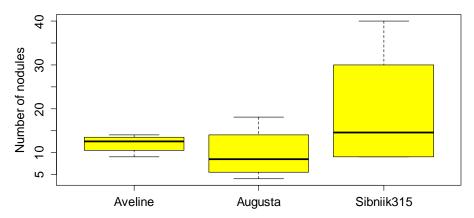
Two early/very early varieties from Germany (Augusta, Aveline) and one regional Siberian (Sibniik315) were grown in completely randomized block design with four repetitions in two variants: with and without *Bradyrhizobium japonicum* inoculation. The treatment was done directly before seeding (15.05.2013) with the peat based two components product "Force48".

Chlorophyll content was measured at the last full developed leaf using a Minolta SPAD-502 device as average value from 30 measurements per plot. The relationship between SPAD values and chlorophyll content is non-linear for soybeans and follows an exponential equation (Markwell et al., 1995). SPAD meter readings as well as leaf chlorophyll content is closely related to leaf nitrogen content (Fritschi and Ray, 2007).

Numbers of nodules were counted on carefully digged out roots at randomly collected plants from each plot.

## Results and discussion

The number of nodules has shown significant differences between inoculated and control among all varieties. No plant of the control group was infected by bacterias, but at every inoculated plant nodules developed (Figure 2). At roots from the regional Siberian variety the highest number of nodules was counted. End of July (full flowering) soy plants from Aveline had from 9 to 14, those from Augusta between 4 and 18 while Sibniik315 showed 9 to 40 nodules.



**Figure 2:** Number of nodules at inoculated varieties from Germany (Aveline, Augusta) and Siberia (Sibniik315) at full flowering (BBCH 65) on July 20<sup>th</sup> 2013.

At all plants the nodules were located close to the main root with diameters between 3-5 mm at that stage of development (Figure 3B). The color of the nodules was light red in the inside which means that they were slightly active (Figure 3C).

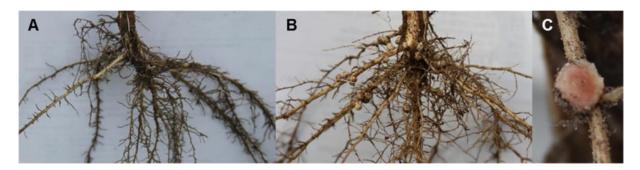
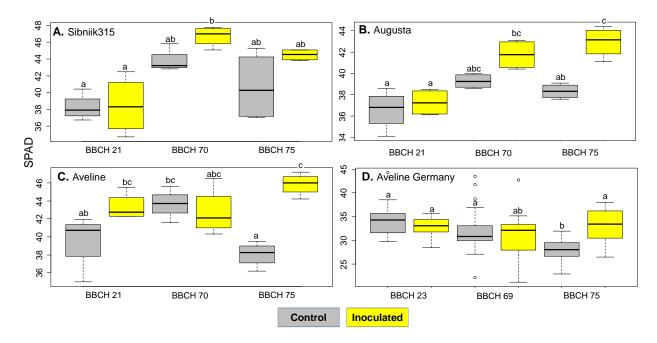


Figure 3: Roots from two Sibniik315 plants without (A) and with (B) inoculation, sliced nodule (C) during full flowering (BBCH 65).

According to the amount of nodules among the inoculated variants also the SPAD-502 chlorophyll meter readings showed differences. SPAD values tended to be higher with inoculation than in the untreated control group (Figure 4). Significant differences appeared at the third measurement (latest observed stage) during mid of seed development. In average Aveline showed the highest increase in SPAD values as well in Siberia (+20.3%) as in Germany (+19.1%) compared to control, followed by Augusta (+12.7%) and Sibniik315 (+9.8%) in Siberia.



**Figure 4:** SPAD meter readings from 3 soybean varieties in Siberia (A,B,C) and Germany (D) at beginning of side shoot formation (BBCH 21/23), end of flowering (BBCH 69/70) and mid of seed development (BBCH 75).

Comparable with the result reported by (Herridge and Rose, 2000) the development of leaf chlorophyll content was specific for every variety. No significant differences among inoculated plants at all three observations could be measured in Aveline at both locations (Figure 4 C,D yellow). In contrast SPAD-values of inoculated Augusta and Sibniik315 increased significant over time (BBCH 21-75), but no significant differences occur without treatment (Figure 4 A,B).

The highest Sibniik315 SPAD value was noticed at the second measurement (BBCH 70, inoculated) which decreased slightly in BBCH 75 (Figure 4 A) as well as in the control. This may imply a better adaption of the Siberian variety to the local climate condition (short vegetation period) than the German breedings because relocation effects from vegetative to generative plant organs occur earlier. Nevertheless inoculated Sibniik315 showed the highest number of nodules, SPAD measurements were less distinctive (not significant) higher compared to control.

In addition to the short vegetation period in Siberia also soil temperatures play an important role for plant physiological processes of nodulating legumes.

Date [dd/mm/yy]	ВВСН	Soiltemperature [° C]
12/05/13	before seeding	10.0 ±1.0
28/0513	09	$14.6 \pm 1.1$
19/06/13	13	$21.3 \pm 2.5$
08/08/13	74	$20.0 \pm 1.0$

Table 1: Soil temperature in 12-20cm depth. Average of 3 measurements ± standard derivation

Optimal conditions for symbiotic fixation are soil temperatures between 25 to  $30^{\circ}$ C (Lynch and Smith, 1993; Zhang et al., 1996). In contrast the measured soil temperatures in Kuchak were only  $\pm~20^{\circ}$ C until BBCH 74 (Table 1) and may cause the slow increase of symbiotic activity.

### Conclusion

Inoculation of soybeans with *Bradyrhizobium japonicum* is necessary otherwise no nodules for atmospheric nitrogen fixation will be developed under Siberian conditions. Due to low soil temperatures below the optimal range for symbiotic activity the significant effect of inoculation shows up at a late stage of development (BBCH 75). Therefore the advantages of inoculation may be limited by Siberian climate conditions.

Local varieties are better adapted to local conditions, but genotypes from other regions must be used to improve the local breedings.

After harvesting and analyzing the varieties in October final conclusions will be possible.

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