https://doi.org/10.17221/346/2019-PSE

Effect of sowing density on grain yield, protein and oil content and plant morphology of soybean (*Glycine max* L. Merrill)

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Citation: Sobko O., Hartung J., Zikeli S., Claupein W., Gruber S. (2019): Effect of sowing density on grain yield, protein and oil content and plant morphology of soybean (*Glycine max* L. Merrill). Plant Soil Environ., 65: 594–601.

Abstract: To find out exactly how sowing density and sowing pattern affect soybean grain yield, quality and its components in non-traditional soybean growing regions, such as Germany, two field trials have been conducted at two locations in Bavaria in 2016 and 2017. The experiments were carried out with four sowing densities (30, 50, 70, 90 seeds/m^2) and four cultivars from different maturity groups (Viola 000, Lissabon 000, ES Mentor 00, Orion 00) as randomized complete block design with four replicates. Almost all evaluated traits varied significantly depending on year × location. There was no interaction between the main factors (cultivar × sowing density). The results revealed that grain yield and height of the first pod increased with increase of sowing density. The number of pods per plant and branching decreased with increasing sowing density. At higher sowing densities at flowering leaf area index was significantly higher than at lower sowing densities. The cv. ES Mentor (00) with 70 seeds/m^2 has proved to be a suitable cultivar in terms of yield and quality in southern Germany (> 3.6 t/ha grain yield and 40% protein).

Keywords: seed quality; climate change; competition; height of first pod; yield structure; harvest index

Soybean is a double use crop with focus on different ingredients (oil and protein) depending on the production area, and the demand. The average oil content in soybean seeds is between 18% and 25%. The by-product of oil extraction is soybean meal with 40–44% crude protein. It is the main source of protein for the food industry and for animal feed (Yilmaz 2003). With the increasing demand for vegetarian foods, soybean protein is increasingly in focus. The crop has high heat demand and a long growing season; for this reason, cultivation is still restricted in some areas of Central and Northern Europe, though it has a long tradition in Asian countries and is frequently grown in South and North America. The interest in soybean production has been growing since several years in Germany, and climate change offers the opportunity to grow the crop on non-traditional locations as well (Hahn and Miedaner 2013). Therefore, the acreage used for soybean production increased rapidly: in 2017 only 19 100 ha were cropped with soybeans in Germany while in 2018 the area increased to 23 900 ha – an increase by 25% in one year (Sojafoedering 2018).

The productivity of soybean is affected by the genetic potential of the cultivar, environmental conditions, and techniques of cultivation (Yilmaz 2003). To increase the yield, taking southern Germany as an example, where the crop heat units (CHU) are not considered to be at optimum levels, it is very important to choose appropriate cultivars and appropriate production systems with improved cultivation methods, including sowing density. Sowing density is a suitable tool to regulate plant growth, crop biomass and yield, and to decrease the production costs with reduced sowing density (Ribeiro et al. 2017). The optimum plant density varies all over the world, and usually ranges between 30 and 60 plants/m^2 (Rahman et al. 2011a). The genotype of soybean from different maturity groups can influence the response to different sowing densities; some cultivars show a better performance for high grain productivity in high crop densities, and others in low densities (Ribeiro et al. 2017). Under the conditions of climate change and unpredictable drought periods, uncertainties of yield occur more often, thus the adjustment of sowing density could control soybean production more efficiently.

The object of this study was to determine the effect of sowing density and cultivar from different maturity groups on the performance of soybean plants. The overall aim was to expand the cultivation of soybean in Germany and other countries with similar climatic conditions.

MATERIAL AND METHODS

Site description. The experiments were conducted at the research station of Saaten Union GmbH on two locations, Grünseiboldsdorf (GSD, 48°29'N, 11°54'E, altitude 440 m a.s.l.) and Landshut (LA, 48°34'N, 12°9'E, altitude 398 m a.s.l.) in south east Germany in 2016 and 2017. These locations are situated in the transitional zone of the maritime climate to a continental climate and are in zone 5 (temperate cool) according global agro-ecological zones (FAO 2018). Weather data for the experimental years were obtained from official meteorological stations nearby (Figure 1).

The experimental design was a randomized complete block design with four replicates. The experimental factors were sowing density in four levels (30, 50, 70 and 90 seeds/m^2) and cultivar in four levels from different maturity groups and growth types (Viola 000, semi determinate; Lissabon 000, determinate; ES Mentor 00, determinate; Orion 00, indeterminate). The plot area was 12.3 m^2, and had 11 rows with a row spacing of 14 cm.

Soil properties and agricultural measures presented in Table 1.

The seeds of all four soybean cultivars were inoculated with HiStick® Soy (2 × 10^9 CFU (colony-forming unit)/g *Bradyrhizobium japonicum*; 400 g/100 kg seeds). The peat formulated inoculant was applied dryly to the seeds immediately before sowing. Since insects and diseases have been below the economic threshold, no chemical insect or disease control were performed, however, control by herbicides was done (Table 1). The harvest of the plots was performed using a plot harvester (HALDRUP C-85, Løgstør, Denmark) with a cutting unit of 1.8 m in width.

Monitoring of plant development. At development stage V5 (five unrolled trifoliate leaf; Fehr et al. 1971) crop density was determined by counting the number of plants of four times the length of one running meter in a randomly selected row. At development stage R2 (at flowering/full bloom) and R7 (beginning of maturity) the leaf area index (LAI) was measured with a plant canopy analyser LAI-2000 from LI-COR (LI-COR, 1992). The measurements were performed under the same degree of sunlight intensity (cloudy or sunny).

At R2 the number of nodules per plant were determined according to the following pattern: six plants per plot were carefully dug out in soil cubes of 20 × 20 × 25 cm. The plants were carefully cleaned in a bucket of water, and the soil was checked for any lost nodules. The primary (on the tap roots) and secondary (on lateral roots) nodules were counted. The plant height was determined at development stage R6 on 10 plants per plot. At the beginning of maturity (R7) the height of the first pod (distance from soil surface) was measured for 10 randomly

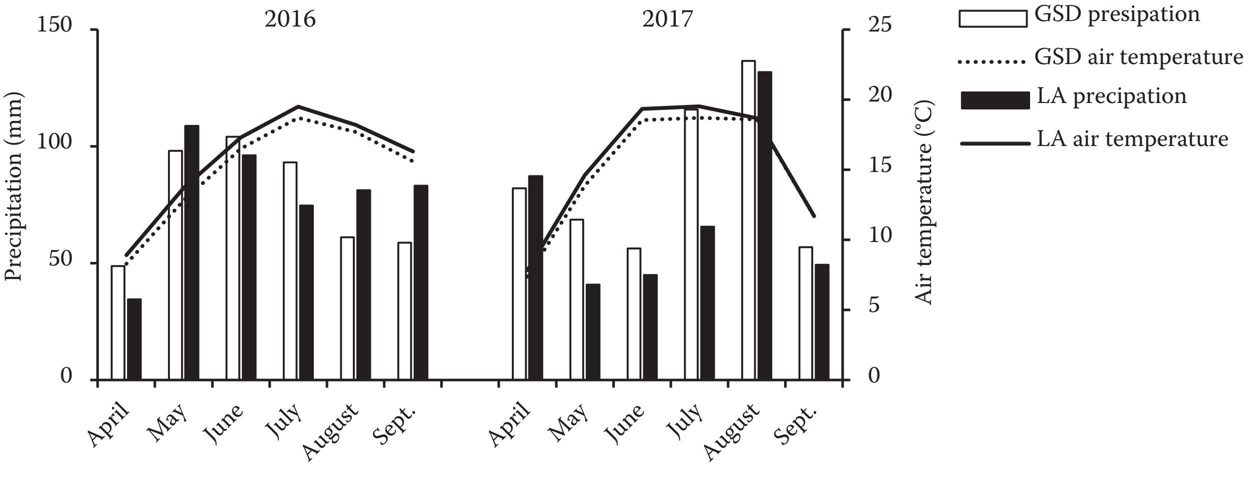

Figure 1. Precipitation and air temperature (average of every month) during the experimental periods (2016 and 2017) at the locations Grünseiboldsdorf (GSD) and Landshut (LA) (Agrometeorology Bayern 2018)

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Table 1. Cultural practices and soil properties for locations Grünseiboldsdorf (GSD) and Landshut (LA)

	2016		2017	
	GSD	LA	GSD	LA
Soil type	L3/Loe (loam loess)	L3/Loe (loam loess)	L2/Loe (loam loess)	L/Mo-b2 (loam moor)
$N_{min\ 0–90\ cm}$ in early spring (kg/ha)	13	120	36	145
Preceding crop	winter wheat (*Triticum aestivum* L.)	winter barley (*Hordeum vulgare* L.)	winter wheat (*T. aestivum* L.)	maize (*Zea mays* L.)
Pre-preceding crop	sugar beet (*Beta vulgaris* L.)	winter wheat (*T. aestivum* L.)	maize (*Z. mays* L.)	winter wheat (*T. aestivum* L.)
Primary tillage^1	mouldboard ploughing (25 cm)			
Seedbed preparation	rotary harrow (28/04/2016)	rotary harrow (12/04/2016)	rotary harrow (28/03/2017)	rotary harrow (18/04/2017 and 06/05/2017)
Seeding^2	02/05/2016	21/04/2016	24/04/2017	06/05/2017
Herbicide application time	Centium 36 C 0.25 L/ha + Sencor WG 0.3 kg/ha + Spektrum 0.8 L/ha			
	05/05/2016	28/04/2016	17/05/2017	17/05/2017
Harvesting date	30/09/2017	13/09/2016 and 30/09/2017	26/09/2017 and 05/10/2017	29/09/2017

^{1}in autumn before soybean cultivation; ^{2}3.5–4.0 cm

picked plants per plot. Additionally, in R7 samples were taken on a sampling area of 0.25 m^2 from each plot to determine the harvest index (HI), the number of pods per plant and the number of seeds per pod. The harvest index was calculated by the formula:

HI = dry grain mass/(dry grain mass + dry mass of soybean) (Spaeth et al. 1984).

At the same time the number of side branches was determined. Plant lodging was evaluated via a visual scoring at R8 from 1 to 9 on plot level (1 = no plant lodging and 9 = total plant lodging).

Post-harvest treatments and laboratory analyses. After harvesting, the samples were cleaned using PFEUFFER MLN (sieve machine with aspiration and deawner, Kitzingen, Germany) and for the determination of thousand kernel mass (TKM), a MARVIN seed analyser (Neubrandenburg, Germany) was used. Oil and protein content in the harvested seed samples were measured in 400 g intact and freshly harvested soybean grains by near infrared reflectance spectroscopy (NIRS) with appropriate calibration from Polytec PSSSHA03-2.1 (Pazdernik et al. 1997).

Data analysis methods. The statistical analysis was done using the PROC MIXED procedure of the SAS system version 9.4M2 (Littell 2004). Normal distribution and homogeneity of variances of residuals were checked graphically. Interaction between sowing densities and cultivars, year and location were fitted. Year, location and year-by-location interaction effects were assumed as random but taken as fixed in the analysis, as expected inter-environmental information is low and the number of levels were only 2 and 4, respectively. A year-by-location-specific error variance was fitted (PROC MIXED). In case of significant *F*-tests, a simple multiple *t*-test (least significant difference (*LSD*)) was performed (Piepho et al. 2003). Additionally, a regression analysis of the main factors was carried out.

RESULTS AND DISCUSSION

The factors sowing density and cultivar had significant effects on yield and some yield components (Table 2). None of the interactions of sowing density × cultivar were significant. However, some parameters were significantly influenced by interaction year-by-location. The exceptions were grain yield, LAI R7, crop density, pods/plant and seeds/pod, HI.

Grain yield. The grain yield across all cultivars and locations was slightly higher in 2017 (3.6 t/ha dry matter (DM)) than in 2016 (3.4 t/ha DM), and yield at both locations was also almost the same in GSD with 4.2 t/ha and 4.0 t/ha in LA (over all years and cultivars; data not shown). The highest grain yield occurred at a sowing density of 90 seeds/m^2 with 4.4 t/ha, and the lowest yield at 30 seeds/m^2 with 3.2 t/ha across locations and years (Figure 2). Only the yield of sowing density of 30 seeds/m^2 di-

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Table 2. Analysis of variance (P-value) for soybean grain yield, yield components, protein and oil content, and other agronomic traits of four soybean cultivars sown with different sowing densities, from field trials in the years 2016 and 2017 in south east Germany

	Grain yield	Protein content	Oil content	TKM	LAI		Height of first pod	Lodging
					R2	R7		
SD	0.0278*	0.1263	0.1294	0.0733	< 0.0001***	0.7008	< 0.0001***	0.0446*
CV	0.7619	0.2534	0.0919	0.0680	0.0065*	0.7536	0.0957	0.0957
SD × CV	0.2706	0.2738	0.7640	0.7977	0.4294	0.6701	0.5622	0.7009
Y × L	0.0603	0.0004**	0.0004**	0.0224*	0.0002***	0.1268	0.0314*	0.0208*
	plant height	primary nodules	secondary nodules	crop density	branching	pods/plant	seeds/pod	HI
SD	0.9911	0.3295	0.9384	0.0268*	0.0324*	0.0152*	0.7528	0.9726
CV	0.0927	0.1134	0.1186	0.6141	0.1056	0.7244	0.1442	0.5121
SD × CV	0.5496	0.0972	0.1410	0.9234	0.3514	0.6363	0.5143	0.4465
Y × L	0.0012**	< 0.001***	0.0009***	0.0002	0.0406*	0.0071	0.2613	0.0086

$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; SD – sowing density; CV – cultivar; Y – year; L – location; TKM – thousand kernel mass; LAI – leaf area index; HI – harvest index

ffered significantly from the other sowing densities, although an increasing trend was visible with higher yields at higher sowing densities.

The average grain yield level in 2017 was 2.7 t/ha in Germany (Sojafoedering 2019). All plot yields were higher than this average (Figure 2). Soybean is known for a high phenotypic plasticity (Ribeiro et al. 2017). The maximal soybean grain yield can be achieved if the plant density supports the interception of 95% solar radiation in R5 (at the beginning of seed filling) (Rahman et al. 2011a). This cannot fully explain the declining yields at low sowing density in the current experiment. The standard sowing densities in Germany with 50 to 70 seeds/m^2 depend on the maturity group (Hahn and Miedaner 2013).

Protein and oil content. Protein and oil content were not affected by sowing density or cultivar (Table 3). This is consistent with the results of study Popovic et al. (2012), which imply that the environment is the dominant factor affecting protein content rather than the genetics.

The protein content rose slightly with an increase of sowing density for all cultivars over all locations by trend. At 90 and 70 seeds/m^2 the protein content was approximately 1.0–1.4 percentage points higher than at 30 and 50 seeds/m^2 over all cultivars. Similar

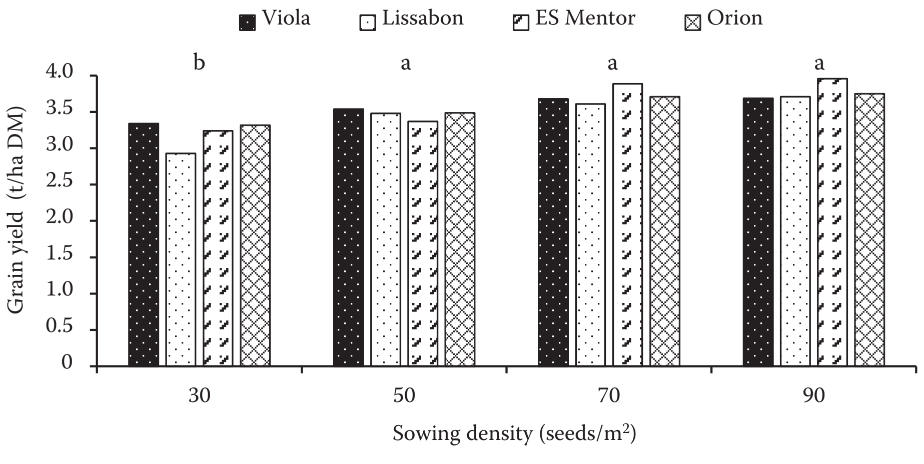

Figure 2. Soybean grain yield (t/ha dry matter (DM)) of the cvs. Viola, Lissabon, ES Mentor and Orion as effect of sowing densities, average of the years 2016 and 2017 and locations Grünseiboldsdorf and Landshut; least significant different (LSD) = 3.0 t/ha DM; no significant differences of values with same letters, $P \leq 0.05$

Table 3. Seed protein and oil content of soybean, means of four cultivars (Viola, Lissabon, ES Mentor, Orion) depending on different sowing densities (A), and means of four sowing densities (30, 50, 70, 90 seeds/m^2) depending on cultivar (B), over two trial years (2016, 2017) and two locations (Grünseiboldsdorf, Landshut)

	Protein content	Oil content
	(% dry matter)	
Sowing density (seeds/m^2) (A)		
30	39.0	20.0
50	39.5	19.7
70	40.0	19.6
90	40.2	19.5
LSD	1.3	0.6
Cultivar (B)		
Viola 000	40.1	19.7
Lissabon 000	38.5	20.3
ES Mentor 00	40.9	19.1
Orion 00	39.4	19.7
LSD	3.0	0.9

LSD – least significant different

results were reported by Boroomandan et al. (2009) and Rahman et al. (2011b). The opposite trend occurred for oil content, which decreased along with higher sowing density. Our research confirmed the negative correlation between protein and oil content (R = 0.88, data not shown) as described by Popovic et al. (2012) and De Luca et al. (2014).

Leaf area index. Sowing density and cultivar significantly affected the LAI at flowering (R2) but not significantly at the beginning of maturity (R7; Figure 3). LAI at low sowing densities was significantly lower at flowering (R2) than at high sowing densities (Figure 3), with a difference of approximately 0.3.

By trend, leaf area index at R2 was positively correlated with sowing density and the higher number of plants as also shown by Weber et al. (1966), Herbert and Litchfield (1984), Wells (1991) and Rahman et al. (2011a). The morphology of the soybean plants, mainly the number of pods and leaves in particular, can be actively changed by altering the sowing density (Egli 1993). At high sowing densities, due to competition, the leaves can become smaller, but their numbers remain high, allowing for greater accumulation of dry matter (Weber et al. 1966, Herbert and Litchfield 1984). A higher LAI results in higher photosynthetic capacity at flowering and grain filling, and finally, in a higher grain yield at higher sowing densities (Wells 1991). The tested cultivars differed in LAI at flowering (R2). The highest LAI (2.5) at flowering was measured for cv. ES Mentor 00, and at the lowest (2.0) for cv. Viola 000. Cvs. Lissabon 000 and Orion 00 both had a LAI of 2.2 and differed significantly from ES Mentor 00. All cultivars varied in growth type, therefore, they may form different leaf canopies as described by BAES (2018). As the development and productivity of the nodes at the main stem in the determinate cultivars ended earlier than that of indeterminate genotypes, this could explain why

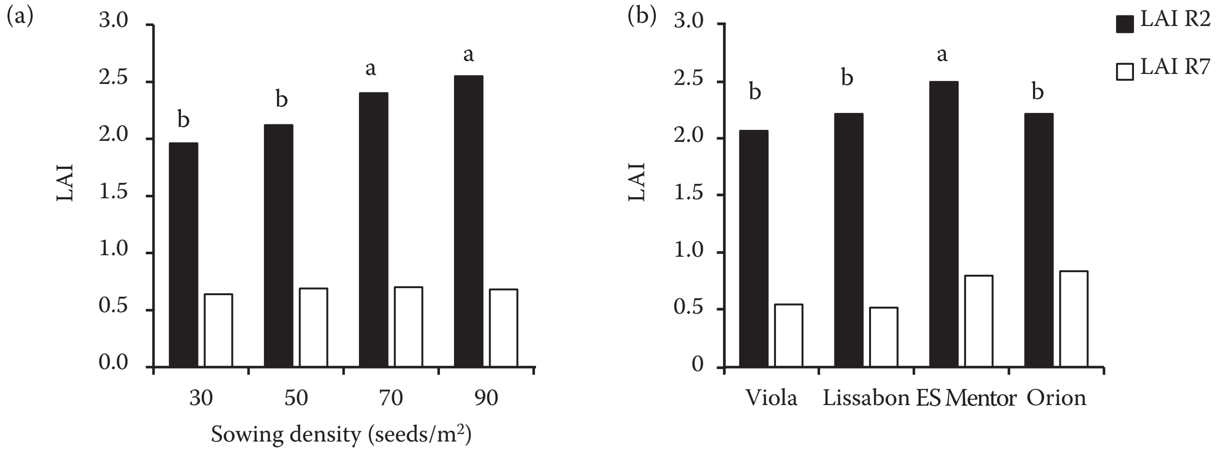

Figure 3. Leaf area index (LAI) (development stages R2 and R7) of soybean, means of years (2016, 2017) and of two locations (Grünseiboldsdorf, Landshut), depending on sowing density (a) across four cultivars (Viola, Lissabon, ES Mentor, Orion), and on cultivars (b) across four sowing densities (30, 50, 70, 90 seeds/m^2); *LSD* (least significant different) LAI R2 (a): 0.09, *LSD* LAI R2 (b): 0.24; no significant differences between values with same letters of R2 at $P \leq 0.05$; no significant differences at R7

https://doi.org/10.17221/346/2019-PSE

Table 4. Morphology traits of soybean at different sowing densities

Sowing density (seeds/m^2)	Height of first pod (cm)	Plant height (cm)	Lodging (rating)*
30	9.4^{d}	83.7	2.7^{b}
50	11.4^{c}	84.7	2.9^{b}
70	12.1^{b}	85.2	3.2^{ab}
90	13.4^{a}	84.6	3.6^{a}
LSD	0.6	ns	0.6

Means of four cultivars (Viola, Lissbon, ES Mentor, Orion), two trial years (2016, 2017) and two locations (Grünseiboldsdorf and Landshut); letters show significant differences at $P \leq 0.05$. *1 – no lodging; 9 – total lodging; *LSD* – least significant different; ns – not significant

Table 5. Average yield structure and crop density by different sowing densities

Sowing density (seeds/m^2)	Crop density (plants/m^2)	Pods/plant (*n*)	TKM (g DM)	Number of branches (*n*)
30	30.9^{c}	30.3^{a}	193.0	2.8^{a}
50	47.5^{bc}	23.0^{b}	198.4	2.2^{ab}
70	61.6^{ab}	18.4^{bc}	199.7	1.5^{b}
90	71.8^{a}	15.6^{c}	202.0	1.4^{b}
LSD	20.9	5.3	ns	0.8

Means of four different cultivars (Viola, Lissabon, ES Mentor, Orion), two trial years (2016, 2017) and both location (Grünseiboldsdorf, Landshut); lower case letters show significant differences at $P < 0.05$. TKN – thousand kernel mass; DM – dry matter; *LSD* – least significant different; ns – not significant

cv. Lissabon 000 had higher LAI at flowering than cv. Viola 000 (Weber et al. 1966, Herbert and Litchfield 1984). Cv. ES Mentor 00 has determinate growth combined with a dense canopy, which could explain the high LAI at R2. This is probably reflected in the highest grain yield of cv. ES Mentor at sowing densities 50, 70 and 90 seed/m^2 compared to the other cultivars of the trial.

LAI at physical maturity (R7) was almost identical at different sowing densities (0.7 in average). LAI of late maturity cvs. such ES Mentor 00 and Orion 00 was higher than early cvs. Viola 000 and Lissabon 000, similar to the results of Weber et al. (1966) and Egli (1993).

Plant morphology. To avoid cutting losses at mechanical harvest it is necessary that the first pod (lowest insertion) has a large distance to the soil surface. This morphological parameter is dependent on the genetic structure of the soybean cultivar, precipitation level and cultivation technology (Yilmaz 2003). In our study, the height of the first pod was significantly affected by plant density and not affected by cultivar (Tables 2 and 4).

Across all locations and years, the height of the first pod increased with increasing sowing density and ranged from 9.4 cm at 30 seeds/m^2 to 13.4 cm at 90 seeds/m^2, similar to Yilmaz (2003) and Epler and Staggenborg (2008). A typical harvest cutting height for soybean is between 7.5 and 12.5 cm above the soil surface (Epler and Staggenborg 2008, Mehmet 2008). Therefore, at high sowing densities harvest losses are not likely to occur. In contrast, the probability of harvest losses by low sowing density was higher, since the height of first pod was just over 9 cm. The common sowing density in Germany is 55 germinable seeds/m^2 (Sojafoedering 2019). The harvest losses could be avoided by increasing of sowing density on the locations with enough water availability.

Plant height and lodging were not affected by cultivar. The lodging was affected significantly by sowing density (Tables 2 and 4). With increasing sowing density, plant height increased, and along with it the risk of lodging. The comparatively high lodging of some cultivars at 90 seeds/m^2 (data not shown) which occurred at the highest sowing density did not reduce yield, similar to Boquet (1990).

Nodulation. The number of nodules was not influenced by sowing density or cultivar (Table 2). The number of primary nodules tended to be higher at low sowing densities compared to higher sowing densities across all cultivars (data not shown). The

Table 6. Number of seeds per pod and harvest index (HI) of soybean as effect of different sowing densities and of cultivar, average over two trial years (2016, 2017) and two locations in south east Germany

Sowing density (seeds/m^2)	Seeds/pod	HI	Cultivar	Seeds/pod	HI
30	2.28	0.56	Viola	2.27	0.54
50	2.23	0.56	Lissabon	2.29	0.53
70	2.24	0.55	ES Mentor	2.26	0.56
90	2.18	0.55	Orion	2.10	0.58
LSD	0.27	0.07	*LSD*	0.19	0.14

LSD – least significant different

number of secondary nodules was almost identical at all sowing densities. There were no significant differences in the number of primary and secondary nodules among the different cultivars across all sowing densities (data not shown).

Yield and yield components. Grain yield is formed by several components. Crop density, number of pods/plant and number of branches were significantly influenced by sowing density (Tables 2 and 5), but TKM, one of the key components, remained unaffected by sowing density and cultivar in our experiments.

At lower sowing densities (30 and 50 seeds/m^2), the number of established plants per square meter was more similar than higher sowing densities. A reason might be that competition between plants increases with increasing sowing density (Yunusa and Ikawelle 1990), thus not all seeds and seedlings developed into a plant at higher sowing densities.

The number of branches/plant ranged between 1.4 at sowing density of 90 seeds/m^2, and 2.8 at sowing density of 30 seeds/m^2 (Table 5). At high sowing density, the space for each individual plant was lower compared to low sowing density, and the plants produced fewer or even no side branches, which also reduced the number of pods per plant (Table 5). It cannot be firmly stated whether the number of branches was the only determinant of the number of pods per plant in the trial. Some studies from South of Brazil (about 1100 m altitude) show lower grain yields at high sowing densities (45 seeds/m^2) compared to low densities (25 seeds/m^2) (Spader and Deschamps 2015, Souza et al. 2016). In contrast, our trials have shown the highest grain yield at the highest sowing density (Figure 1). Clearly, the high number of plants per area compensated the lower number of branches and of pods per plant in terms of grain yield. The highest number of pods/plant was obtained at 30 seeds/m^2, with > 30 pods per plant (Table 5) and showed a negative correlation with the sowing density. Several studies, including our own trial, show that soybean is a flexible crop which produces grain yield by several constellations of the yield components (Epler and Staggenborg 2008, Mehmet 2008, Ferreira et al. 2016, Ribeiro et al. 2017).

TKM was not significantly affected by sowing density (Table 5) and ranged between 193 g (30 seeds/m^2) and 202 g (90 seeds/m^2). Mehmet (2008) and Spader and Deschamps (2015) report that the TKW tended to decline with increasing sowing density.

The number of seeds per pod and harvest index tended to decrease with increasing sowing density though not significantly. Regardless of sowing density and cultivar, the pods had an average of 2.23 seeds (Table 6).

According to Spader and Deschamps (2015), the number seeds/pod is mainly genetically determined, though the results of the current study show at the very least, a trend of the effect of cultivation techniques on the number of seeds per pod. According to research results of Herbert and Litchfield (1984) and Mehmet (2008) harvest index is stable characteristic within a cultivar and is not affected by sowing density, similar to our results.

Acknowledgement

We thank Saaten Union GmbH for the opportunity to carry out the trials, and for any support. Special thanks go to Franz Xaver Zellner, who has supported the field trials.

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Received on June 20, 2019
Accepted on October 7, 2019