

*Article*

# Effect of Nitrogen Fertilisation and Inoculation with *Bradyrhizobium japonicum* on the Fatty Acid Profile of Soybean (*Glycine max* (L.) Merrill) Seeds

Ewa Szpunar-Krok, Anna Wodołowska-Grabowska, Dorota Bobrecka-Jamro, Marta Jańczak-Pieniążek, Andrzej Kotecki and Marcin Kozak

**Table S1.** FA composition of *G. max* seeds (g FA · 100 g seeds<sup>-1</sup>), mean values for interaction cultivar × fertilisation.

Cultivar	Fertilisation (kg·ha <sup>-1</sup> N)	C18:0	C18:1n9	C18:2n6	C18:3n6	C18:3n3	C14:0	C16:0	C16:1	C20:0	C20:1
Aldana	0	3.17 ± 0.13	20.0 ± 3.07	52.3 ± 1.13	0.079 ± 0.054	8.89 ± 0.61	0.173 ± 0.138	12.7 ± 1.80	0.147 ± 0.027	0.275 ± 0.033	0.189 ± 0.027
	30	3.11 ± 0.21	19.7 ± 2.35	52.5 ± 1.83	0.088 ± 0.047	8.89 ± 0.70	0.167 ± 0.095	12.9 ± 1.25	0.152 ± 0.023	0.249 ± 0.052	0.177 ± 0.045
	60	3.14 ± 0.27	20.3 ± 2.97	51.9 ± 1.87	0.073 ± 0.044	8.78 ± 0.89	0.154 ± 0.103	13.1 ± 0.94	0.170 ± 0.050	0.252 ± 0.027	0.169 ± 0.033
Annushka	0	3.35 ± 0.28	18.3 ± 2.54	53.4 ± 1.22	0.088 ± 0.044	9.07 ± 0.76	0.162 ± 0.085	12.8 ± 1.65	0.134 ± 0.027	0.314 ± 0.080	0.204 ± 0.063
	30	3.26 ± 0.28	17.9 ± 1.88	53.3 ± 1.93	0.088 ± 0.047	9.05 ± 0.86	0.162 ± 0.078	13.6 ± 1.25	0.133 ± 0.021	0.268 ± 0.044	0.164 ± 0.029
	60	3.24 ± 0.23	17.9 ± 1.71	53.1 ± 1.30	0.081 ± 0.051	8.96 ± 0.84	0.153 ± 0.075	13.7 ± 0.75	0.149 ± 0.041	0.265 ± 0.039	0.182 ± 0.036
Cultivar × Fertilisation		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

\*Values are expressed as mean ± SD. Means in a column followed by different letters show significant differences ( $p < 0.05$ ) according to the Tukey test. NS not significant.

**Table S2.** FA composition of *G. max* seeds ( $\text{g FA} \cdot 100 \text{ g seeds}^{-1}$ ), mean values for interaction fertilisation  $\times$  inoculation.

Fertilisation ( $\text{kg} \cdot \text{ha}^{-1} \text{ N}$ )	Inoculated	C18:0	C18:1n9	C18:2n6	C18:3n6	C18:3n3	C14:0	C16:0	C16:1	C20:0	C20:1
0	Without inoculation	3.18 $\pm$ 0.22	18.6 $\pm$ 3.20	52.9 $\pm$ 1.21	0.093 $\pm$ 0.053	8.93 $\pm$ 0.66	0.186 $\pm$ 0.099	13.5 $\pm$ 1.57	0.146 $\pm$ 0.033	0.271 $\pm$ 0.043	0.180 $\pm$ 0.043
	HiStick®Soy	3.33 $\pm$ 0.19	19.7 $\pm$ 3.24	52.9 $\pm$ 1.43	0.075 $\pm$ 0.033	8.97 $\pm$ 0.78	0.126 $\pm$ 0.094	12.2 $\pm$ 1.91	0.136 $\pm$ 0.031	0.322 $\pm$ 0.090	0.213 $\pm$ 0.060
	Nitra-gina	3.27 $\pm$ 0.28	19.1 $\pm$ 2.45	52.7 $\pm$ 1.77	0.081 $\pm$ 0.061	9.04 $\pm$ 0.69	0.190 $\pm$ 0.142	12.5 $\pm$ 1.52	0.139 $\pm$ 0.017	0.293 $\pm$ 0.042	0.197 $\pm$ 0.038
30	Without inoculation	3.19 $\pm$ 0.28	18.5 $\pm$ 1.49	52.8 $\pm$ 1.22	0.079 $\pm$ 0.050	9.00 $\pm$ 0.77	0.158 $\pm$ 0.089	13.9 $\pm$ 1.23	0.151 $\pm$ 0.021	0.258 $\pm$ 0.068	0.177 $\pm$ 0.047
	HiStick®Soy	3.23 $\pm$ 0.23	19.2 $\pm$ 3.02	53.2 $\pm$ 1.56	0.106 $\pm$ 0.056	9.00 $\pm$ 0.85	0.170 $\pm$ 0.066	12.5 $\pm$ 1.53	0.131 $\pm$ 0.015	0.272 $\pm$ 0.036	0.173 $\pm$ 0.030
	Nitragina	3.13 $\pm$ 0.27	18.8 $\pm$ 2.31	52.7 $\pm$ 1.93	0.076 $\pm$ 0.025	8.92 $\pm$ 0.81	0.166 $\pm$ 0.107	13.3 $\pm$ 0.52	0.146 $\pm$ 0.030	0.245 $\pm$ 0.036	0.162 $\pm$ 0.038
60	Without inoculation	3.22 $\pm$ 0.19	19.2 $\pm$ 2.73	52.8 $\pm$ 1.30	0.067 $\pm$ 0.025	9.02 $\pm$ 0.79	0.122 $\pm$ 0.054	13.2 $\pm$ 1.18	0.147 $\pm$ 0.040	0.261 $\pm$ 0.027	0.171 $\pm$ 0.020
	HiStick®Soy	3.19 $\pm$ 0.21	19.3 $\pm$ 3.24	51.9 $\pm$ 2.39	0.086 $\pm$ 0.061	8.71 $\pm$ 1.08	0.201 $\pm$ 0.090	13.5 $\pm$ 0.60	0.177 $\pm$ 0.058	0.253 $\pm$ 0.031	0.179 $\pm$ 0.039
	Nitragina	3.16 $\pm$ 0.27	18.8 $\pm$ 2.28	52.8 $\pm$ 2.14	0.080 $\pm$ 0.051	8.88 $\pm$ 0.75	0.137 $\pm$ 0.102	13.5 $\pm$ 0.89	0.154 $\pm$ 0.037	0.261 $\pm$ 0.044	0.177 $\pm$ 0.043
Fertilisation $\times$ Inoculation		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

\*Values are expressed as mean  $\pm$  SD. Means in a column followed by different letters show significant differences ( $< 0.05$ ) according to the Tukey test. NS not significant.

**Table S3.** FA composition of *G. max* seeds ( $\text{g FA} \cdot 100 \text{ g seeds}^{-1}$ ), mean values for interaction inoculation  $\times$  years.

Inoculated	Years	C18:0	C18:1n9	C18:2n6	C18:3n6	C18:3n3	C14:0	C16:0	C16:1	C20:0	C20:1
Without inoculation	2016	3.20 $\pm$ 0.13	17.1 $\pm$ 1.39	54.4 $\pm$ 0.53	0.123 $\pm$ 0.037	9.21 $\pm$ 0.30	0.175 $\pm$ 0.037	13.8 $\pm$ 0.42	0.124 $\pm$ 0.016	0.263 $\pm$ 0.017	0.167 $\pm$ 0.022
	2017	3.38 $\pm$ 0.17	18.7 $\pm$ 0.78	52.5 $\pm$ 0.27	0.099 $\pm$ 0.040	8.37 $\pm$ 0.28	0.207 $\pm$ 0.061	13.5 $\pm$ 0.77	0.148 $\pm$ 0.014	0.295 $\pm$ 0.038	0.207 $\pm$ 0.045
	2018	2.91 $\pm$ 0.14	21.8 $\pm$ 2.94	51.8 $\pm$ 1.10	0.050 $\pm$ 0.030	8.42 $\pm$ 0.33	0.171 $\pm$ 0.100	12.9 $\pm$ 1.46	0.144 $\pm$ 0.032	0.247 $\pm$ 0.078	0.168 $\pm$ 0.035
	2019	3.30 $\pm$ 0.10	17.5 $\pm$ 0.82	52.7 $\pm$ 0.79	0.048 $\pm$ 0.011	9.94 $\pm$ 0.19	0.069 $\pm$ 0.056	14.0 $\pm$ 0.49	0.176 $\pm$ 0.037	0.248 $\pm$ 0.043	0.163 $\pm$ 0.033
HiStick®Soy	2016	3.14 $\pm$ 0.11	17.1 $\pm$ 1.22	54.9 $\pm$ 0.74	0.072 $\pm$ 0.032	9.49 $\pm$ 0.19	0.145 $\pm$ 0.016	13.3 $\pm$ 0.28	0.124 $\pm$ 0.011	0.253 $\pm$ 0.010	0.165 $\pm$ 0.006
	2017	3.43 $\pm$ 0.13	18.8 $\pm$ 0.79	52.1 $\pm$ 0.70	0.127 $\pm$ 0.062	8.36 $\pm$ 0.21	0.258 $\pm$ 0.043	13.5 $\pm$ 0.48	0.156 $\pm$ 0.025	0.305 $\pm$ 0.034	0.212 $\pm$ 0.025
	2018	3.06 $\pm$ 0.18	23.9 $\pm$ 2.24	50.7 $\pm$ 1.57	0.090 $\pm$ 0.062	7.90 $\pm$ 0.62	0.168 $\pm$ 0.091	11.1 $\pm$ 1.15	0.158 $\pm$ 0.080	0.320 $\pm$ 0.050	0.225 $\pm$ 0.059
	2019	3.38 $\pm$ 0.16	17.8 $\pm$ 0.92	53.0 $\pm$ 0.92	0.067 $\pm$ 0.025	9.82 $\pm$ 0.36	0.093 $\pm$ 0.082	13.0 $\pm$ 1.14	0.154 $\pm$ 0.017	0.250 $\pm$ 0.048	0.150 $\pm$ 0.034
Nitragina	2016	3.13 $\pm$ 0.11	17.1 $\pm$ 1.16	54.4 $\pm$ 0.87	0.100 $\pm$ 0.042	9.36 $\pm$ 0.19	0.160 $\pm$ 0.039	13.4 $\pm$ 0.35	0.130 $\pm$ 0.019	0.258 $\pm$ 0.016	0.175 $\pm$ 0.010
	2017	3.36 $\pm$ 0.18	18.5 $\pm$ 0.79	51.9 $\pm$ 0.52	0.118 $\pm$ 0.053	8.34 $\pm$ 0.31	0.289 $\pm$ 0.121	13.5 $\pm$ 0.37	0.164 $\pm$ 0.026	0.300 $\pm$ 0.032	0.215 $\pm$ 0.045
	2018	2.88 $\pm$ 0.18	22.1 $\pm$ 1.77	51.6 $\pm$ 1.60	0.050 $\pm$ 0.025	8.31 $\pm$ 0.47	0.146 $\pm$ 0.084	12.5 $\pm$ 1.56	0.147 $\pm$ 0.040	0.262 $\pm$ 0.062	0.183 $\pm$ 0.043
	2019	3.38 $\pm$ 0.24	18.1 $\pm$ 1.03	53.1 $\pm$ 1.44	0.048 $\pm$ 0.010	9.77 $\pm$ 0.29	0.062 $\pm$ 0.077	13.1 $\pm$ 1.44	0.145 $\pm$ 0.020	0.243 $\pm$ 0.039	0.143 $\pm$ 0.026
Inoculation $\times$ Years		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

\*Values are expressed as mean  $\pm$  SD. Means in a column followed by different letters show significant differences ( $p < 0.05$ ) according to the Tukey test. NS not significant.

**Table S4.** Content of SFA, UFA, MUFA and PUFA (g FA·100 g seeds<sup>-1</sup>) as well as the ratio of SFA/UFA and C18:2n6/C18: 3n3 in *G. max* seeds, mean values for interaction cultivar × fertilisation.

Cultivar	Fertilisation (kg·ha <sup>-1</sup> N)	SFA	MUFA	UFA	PUFA	SFA/UFA	C18:2n6/C18:3n3
Aldana	0	17.6 ± 1.40	20.5 ± 3.06	82.6 ± 1.81	61.7 ± 2.47	0.21 ± 0.03	5.91 ± 0.39
	30	17.6 ± 1.51	20.2 ± 2.38	81.6 ± .99	61.3 ± 2.03	0.23 ± 0.03	5.92 ± 0.47
	60	17.8 ± 1.18	20.8 ± 3.00	81.9 ± 1.22	61.6 ± 1.78	0.22 ± 0.02	5.96 ± 0.50
Annushka	0	17.8 ± 1.96	18.8 ± 2.58	81.1 ± 1.72	62.4 ± 2.31	0.23 ± 0.03	5.91 ± 0.40
	30	18.4 ± 1.34	18.4 ± 1.88	81.3 ± 1.45	62.8 ± 1.81	0.23 ± 0.02	5.94 ± 0.41
	60	18.8 ± 1.49	18.4 ± 1.76	81.7 ± 1.71	63.2 ± 1.97	0.22 ± 0.03	5.96 ± 0.48
Cultivar × Fertilisation		NS	NS	NS	NS	NS	NS

\*Values are expressed as mean ± SD. Means in a column followed by different letters show significant differences ( $p < 0.05$ ) according to the Tukey test. NS not significant.

**Table S5.** Content of SFA, UFA, MUFA and PUFA (g FA·100 g seeds<sup>-1</sup>) as well as the ratio of SFA/UFA and C18:2n6/C18: 3n3 in *G. max* seeds, mean values for interaction cultivar × inoculation.

Cultivar)	Inoculated	SFA	MUFA	UFA	PUFA	SFA/UFA	C18:2n6/C18:3n3
Aldana	Without inoculation	17.7 ± 1.91	20.2 ± 2.87	82.1 ± 1.89	61.4 ± 2.42	0.22 ± 0.03	5.90 ± 0.42
	HiStick®Soy	17.1 ± 1.84	20.8 ± 3.23	81.8 ± 1.54	61.3 ± 2.25	0.22 ± 0.02	5.9 ± 0.49
	Nitragina	18.2 ± 1.31	20.4 ± 2.30	82.2 ± 1.80	62.0 ± 1.51	0.22 ± 0.03	5.94 ± 0.44
Annushka	Without inoculation	18.9 ± 1.07	18.2 ± 1.60	81.1 ± 1.30	62.7 ± 1.97	0.23 ± 0.02	5.93 ± 0.46
	HiStick®Soy	18.2 ± 1.99	18.9 ± 2.70	81.8 ± 1.70	62.9 ± 2.01	0.22 ± 0.03	5.97 ± 0.45
	Nitragina	17.9 ± 1.68	18.4 ± 1.79	81.2 ± 1.83	62.7 ± 2.21	0.23 ± 0.03	5.91 ± 0.38
Cultivar × Inoculation		NS	NS	NS	NS	NS	NS

\*Values are expressed as mean ± SD. Means in a column followed by different letters show significant differences ( $p < 0.05$ ) according to the Tukey test. NS not significant.

**Table S6.** Content of SFA, UFA, MUFA and PUFA (g FA·100 g seeds<sup>-1</sup>) as well as the ratio of SFA/UFA and C18:2n6/C18: 3n3 in *G. max* seeds, mean values for interaction inoculation × years.

Inoculated	Year	SFA	MUFA	UFA	PUFA	SFA/UFA	C18:2n6/C18:3n3
Without inoculation	2016	18.2 ± 0.63	17.5 ± 1.25	81.7 ± 0.79	64.2 ± 0.74	0.22 ± 0.01	5.91 ± 0.15
	2017	19.2 ± 0.90	19.2 ± 0.94	80.2 ± 0.89	64.4 ± 1.15	0.25 ± 0.01	6.28 ± 0.23
	2018	16.8 ± 1.49	22.3 ± 1.24	83.0 ± 2.45	64.4 ± 0.98	0.21 ± 0.04	6.16 ± 0.27
	2019	18.9 ± 0.90	17.9 ± 0.9	81.4 ± 0.95	61.2 ± 0.31	0.23 ± 0.02	5.30 ± 0.13
HiStick®Soy	2016	17.5 ± 0.32	17.5 ± 1.34	81.8 ± 0.39	61.0 ± 0.21	0.22 ± 0.01	5.79 ± 0.07
	2017	19.2 ± 0.94	19.3 ± 0.84	80.3 ± 0.72	60.9 ± 0.61	0.25 ± 0.01	6.23 ± 0.23
	2018	15.8 ± 1.76	24.5 ± 1.44	83.3 ± 2.11	59.3 ± 2.15	0.20 ± 0.03	6.44 ± 0.36
	2019	18.2 ± 1.36	18.3 ± 0.66	81.6 ± 1.01	59.9 ± 2.54	0.23 ± 0.02	5.40 ± 0.14
Nitragina	2016	17.9 ± 0.48	17.5 ± 1.15	82.0 ± 0.53	61.1 ± 1.55	0.22 ± 0.01	5.82 ± 0.06
	2017	19.5 ± 0.49	19.1 ± 0.48	80.4 ± 0.84	63.4 ± 0.89	0.24 ± 0.01	6.23 ± 0.21
	2018	16.8 ± 1.83	22.5 ± 1.77	83.6 ± 2.32	63.1 ± 1.05	0.20 ± 0.03	6.22 ± 0.41
	2019	18.0 ± 1.35	18.5 ± 1.19	81.0 ± 1.58	62.9 ± 1.76	0.23 ± 0.02	5.44 ± 0.11
Inoculation × Years		NS	NS	NS	NS	NS	NS

\*Values are expressed as mean ± SD. Means in a column followed by different letters show significant differences ( $p < 0.05$ ) according to the Tukey test. NS not significant.