

Endophyte Inoculation and Elevated Potassium Supply on Productivity, Growth and Physiological Parameters of Spring Barley (*Hordeum vulgare L.*) Genotypes Over Contrasting Seasons

Dominik Bleša, Pavel Matušinský, Milan Baláž, Zdeněk Nesvadba and Marta Zavřelová

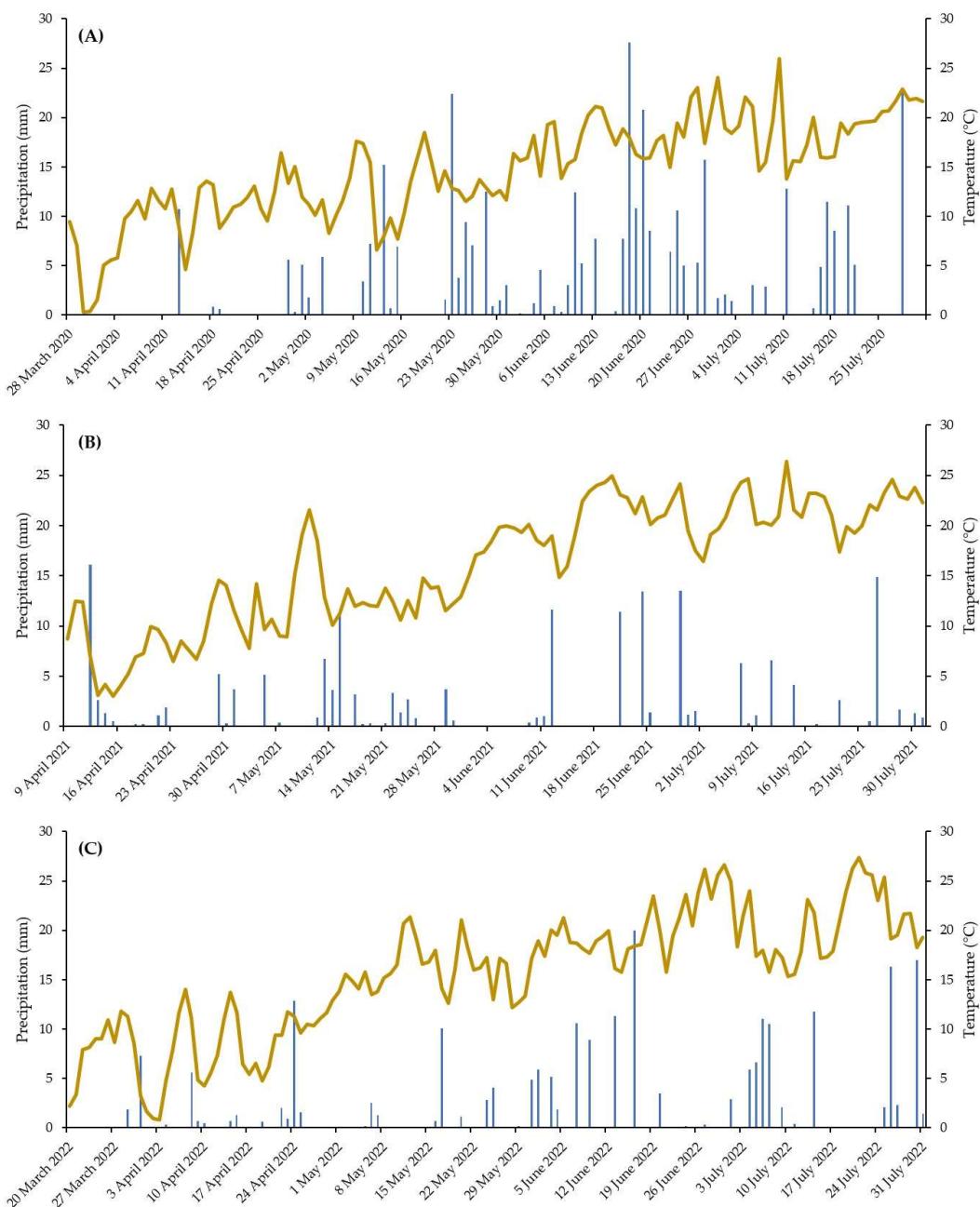
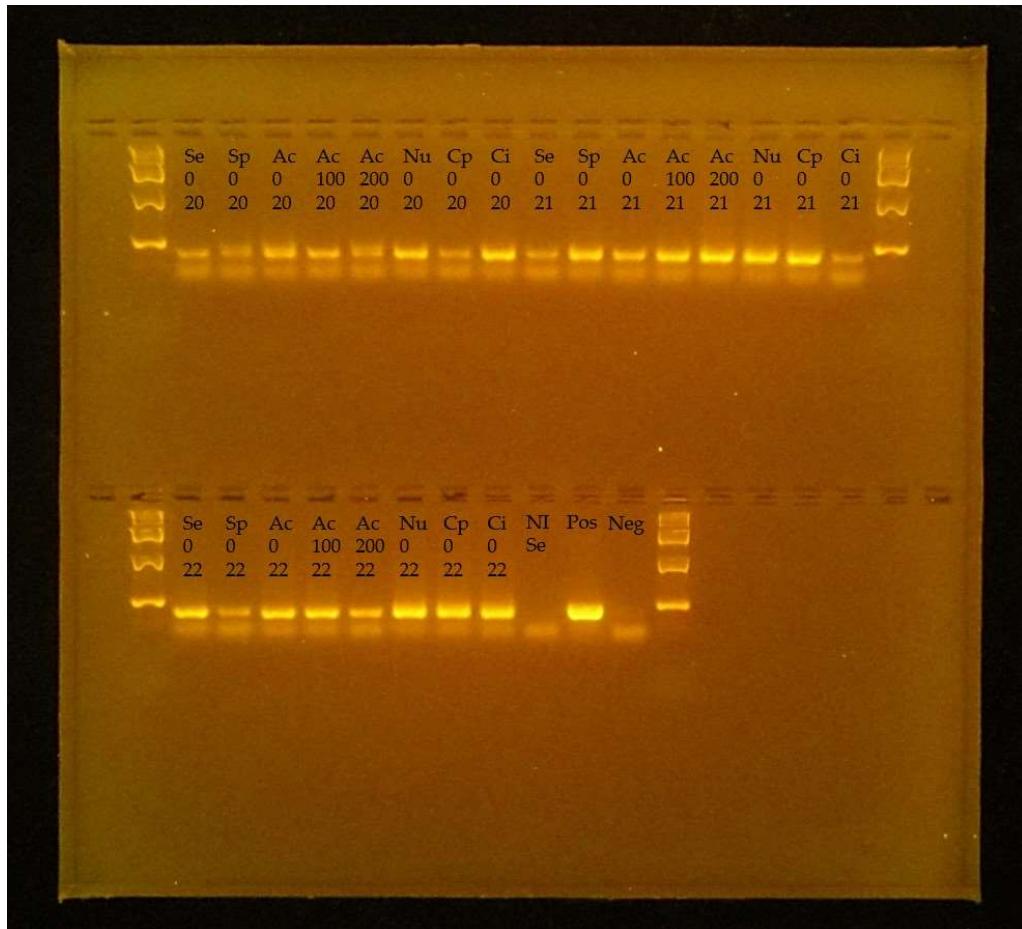


Figure S1. Mean temperatures and precipitation in growing seasons (A) 2020; (B) 2021; (C) 2022.

Table S1. The nutrient content in the soil taken at the experimental site before experiments.

Season	P ($\text{mg}\cdot\text{kg}^{-1}$)	K ($\text{mg}\cdot\text{kg}^{-1}$)	Ca ($\text{mg}\cdot\text{kg}^{-1}$)	Mg ($\text{mg}\cdot\text{kg}^{-1}$)	pH/KCl
2020	70	208	2553	285	5.04
2021	46	137	2355	253	5.27
2022	120	170	2890	153	6.44

**Figure S2.** Electrophoresis gel of the PCR products using *Serendipita indica*-specific primers. Samples were isolated from barley roots in seasons 2020–2022. Se – Sebastian, Sp – Spitfire, Ac – Accordine, Nu – Nutans Afganistan, Cp – CPI 18197, Ci – CI 6388 genotypes; 0, 100, 200 – kg/ha potassium supply; 20 – season 2020, 21 – season 2021, 22 – season 2022; NI – not inoculated; Pos – positive control using fungal DNA, Neg – negative control.

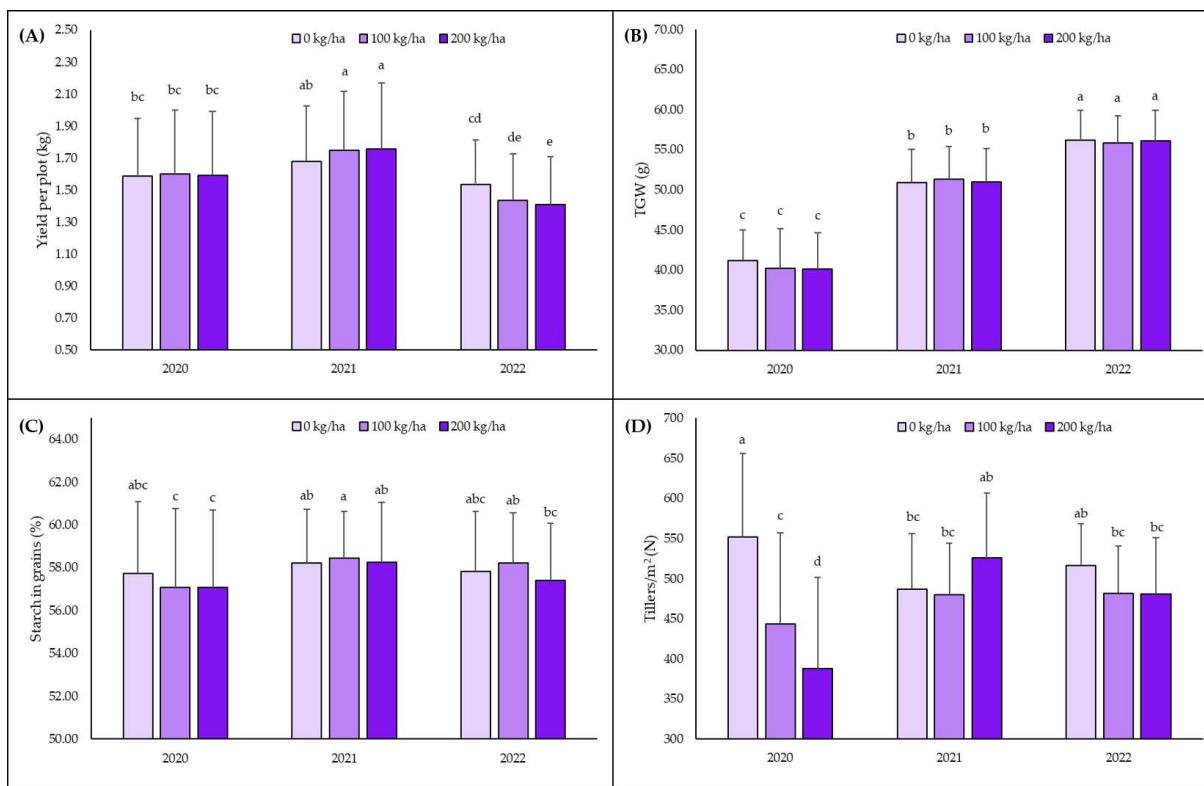


Figure S3. Interaction of fertilization and season on productive parameters and tillering. **(A)** yield per plot; **(B)** thousand grains weight; **(C)** starch in grains; **(D)** tillers. Columns represent means, bars SD ($n = 30$) followed by the same letter if there was no statistical difference according to Tukey $_{0.05}$ test.

Table S2. Interaction of fertilization, inoculation and season factors on the thousand grains weight and plant height. Treatment is in format fertilization (0, 100, or 200 kg of potassium per ha) \times inoculation (NI – not inoculated, *S. indica* – *Serendipita indica*) \times season (2020–2022). Data represent means \pm SD ($n = 12$ for *S. indica*; $n = 18$ for NI) followed by the same letter if there was no statistical difference according to Tukey $_{0.05}$ test for unequal sample sizes.

Treatment	TGW (g)	Plant height (cm)
0 \times NI \times 2020	40.56 \pm 3.82 cd	84.8 \pm 6.5 a
0 \times NI \times 2021	50.91 \pm 3.85 b	75.3 \pm 7.5 cd
0 \times NI \times 2022	56.14 \pm 3.80 a	68.1 \pm 6.6 f
0 \times <i>S. indica</i> \times 2020	41.98 \pm 3.86 c	82.0 \pm 8.8 ab
0 \times <i>S. indica</i> \times 2021	50.80 \pm 4.77 b	75.3 \pm 8.4 cde
0 \times <i>S. indica</i> \times 2022	56.32 \pm 3.69 a	70.5 \pm 6.8 cdef
100 \times NI \times 2020	40.22 \pm 4.96 cd	84.9 \pm 9.8 a
100 \times NI \times 2021	51.24 \pm 4.44 b	76.2 \pm 6.6 bcd
100 \times NI \times 2022	55.88 \pm 3.37 a	71.0 \pm 8.5 cdef
100 \times <i>S. indica</i> \times 2020	40.13 \pm 5.14 cd	88.4 \pm 7.5 a
100 \times <i>S. indica</i> \times 2021	51.34 \pm 3.80 b	75.5 \pm 7.0 bcd
100 \times <i>S. indica</i> \times 2022	55.88 \pm 3.51 a	68.5 \pm 7.9 f
200 \times NI \times 2020	40.69 \pm 4.97 cd	86.3 \pm 7.2 a
200 \times NI \times 2021	50.73 \pm 4.09 b	76.4 \pm 5.7 bc
200 \times NI \times 2022	55.98 \pm 4.00 a	68.6 \pm 8.4 f
200 \times <i>S. indica</i> \times 2020	39.27 \pm 3.81 d	88.0 \pm 8.3 a

$200 \times S. indica \times 2021$	$51.33 \pm 4.43^{\text{b}}$	$76.2 \pm 5.9^{\text{bcd}}$
$200 \times S. indica \times 2022$	$56.34 \pm 3.65^{\text{a}}$	$69.5 \pm 10.0^{\text{ef}}$

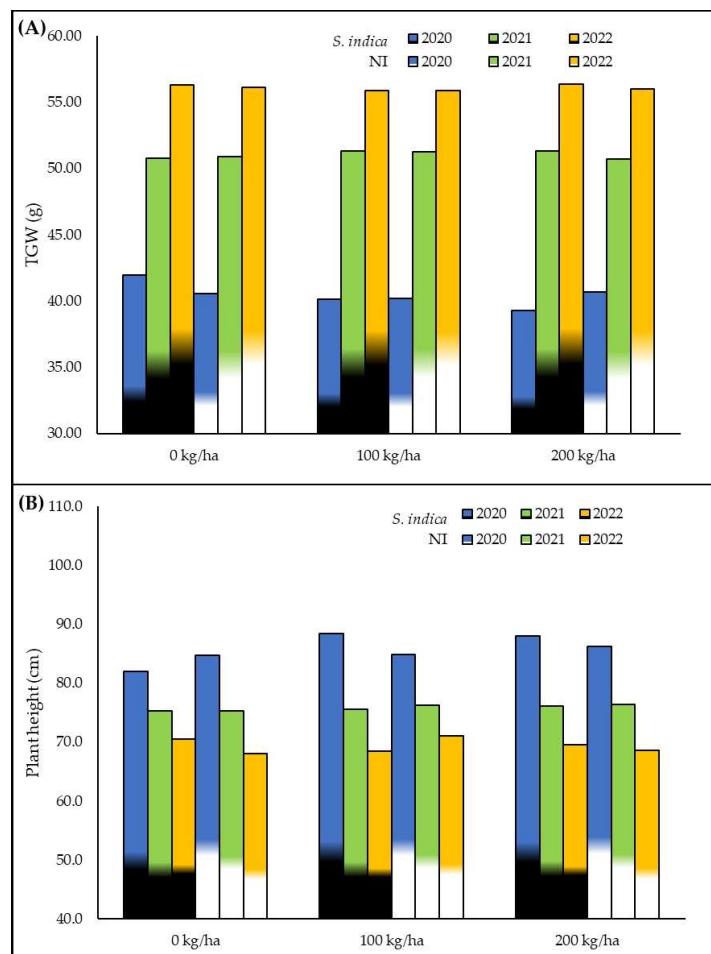


Figure S4. Interaction of fertilization, inoculation, and season factors on the (A) thousand grains weight (TGW) and (B) plant height. Fertilization doses 0, 100, or 200 kg of potassium per ha, season (2020–2022); and inoculation (NI – not inoculated, *S. indica* – *Serendipita indica*). Data represent means ($n = 12$ for *S. indica*; $n = 18$ for NI). Statistical significance and SD values are shown in the Supplementary Table 2.

Table S3. Interaction of genotype, fertilization and season factors on the tillering, plant height and carbon isotope signature ($\delta^{13}\text{C}$). Treatment is in format genotype \times fertilization (0, 100, or 200 kg of potassium per ha) \times season (2020–2022). Data represent means \pm SD ($n = 5$) followed by the same letter if there was no statistical difference according to Tukey_{0.05} test.

Treatment	Tillers/m ² (N)	Plant height (cm)	$\delta^{13}\text{C}$ (‰)
Sebastian \times 0 \times 2020	$608 \pm 74^{\text{ab}}$	$75.4 \pm 2.1^{\text{defghijklmnop}}$	$-29.8 \pm 0.3^{\text{lmnopqr}}$
Sebastian \times 0 \times 2021	$610 \pm 21^{\text{ab}}$	$61.6 \pm 2.9^{\text{stuv}}$	$-29.1 \pm 0.1^{\text{ghijklmno}}$
Sebastian \times 0 \times 2022	$535 \pm 75^{\text{abcdefgh}}$	$60.8 \pm 1.9^{\text{tuv}}$	$-27.6 \pm 0.2^{\text{de}}$
Sebastian \times 100 \times 2020	$602 \pm 56^{\text{abc}}$	$80.6 \pm 3.8^{\text{bcdefghijkl}}$	$-30.2 \pm 0.5^{\text{s}}$
Sebastian \times 100 \times 2021	$573 \pm 27^{\text{abcd}}$	$63.8 \pm 3.0^{\text{rstuv}}$	$-29.1 \pm 0.4^{\text{ghijklmno}}$
Sebastian \times 100 \times 2022	$558 \pm 80^{\text{abcdef}}$	$58.0 \pm 3.5^{\text{v}}$	$-27.8 \pm 0.2^{\text{ef}}$
Sebastian \times 200 \times 2020	$486 \pm 120^{\text{bcdefghijk}}$	$78.6 \pm 1.3^{\text{bcdefghijklm}}$	$-30.3 \pm 0.5^{\text{s}}$
Sebastian \times 200 \times 2021	$642 \pm 14^{\text{a}}$	$65.8 \pm 0.8^{\text{opqrstuvwxyz}}$	$-28.9 \pm 0.0^{\text{ghijkl}}$

Sebastian×200×2022	568 ± 41 abcd	58.6 ± 2.6 uv	-27.7 ± 0.2 de
Spitfire×0×2020	568 ± 144 abcd	85.6 ± 6.4 abcdef	-30.0 ± 0.4 qrs
Spitfire×0×2021	480 ± 33 bcdefghijkl	72.0 ± 2.1 ijklmnopqr	-29.2 ± 0.2 ghijklmnop
Spitfire×0×2022	530 ± 24 abcdefgh	65.0 ± 3.2 pqrstuv	-27.3 ± 0.3 cde
Spitfire×100×2020	430 ± 91 defghijklm	82.4 ± 3.4 bcdefghij	-30.1 ± 0.3 rs
Spitfire×100×2021	529 ± 42 abcdefgh	74.4 ± 2.3 efgijklmnopq	-29.0 ± 0.2 ghijklmn
Spitfire×100×2022	473 ± 33 bcdefghijklm	67.0 ± 5.4 nopqrstuv	-27.2 ± 0.4 cde
Spitfire×200×2020	393 ± 57 ghijklm	86.0 ± 3.9 abcde	-30.1 ± 0.3 rs
Spitfire×200×2021	533 ± 28 abcdefgh	80.0 ± 2.0 bcdefghijkl	-29.0 ± 0.2 ghijklmn
Spitfire×200×2022	473 ± 63 bcdefghijklm	64.8 ± 3.6 qrstuv	-27.4 ± 0.4 cde
Accordine×0×2020	538 ± 116 abcdefgh	86.2 ± 3.0 abcde	-29.8 ± 0.3 nopqrs
Accordine×0×2021	508 ± 21 abcdefghi	81.4 ± 2.2 bcdefghijk	-28.5 ± 0.3 fg
Accordine×0×2022	496 ± 43 abcdefghi	67.2 ± 3.1 nopqrstuv	-27.4 ± 0.3 cde
Accordine×100×2020	542 ± 37 abcdefg	88.0 ± 6.3 abc	-29.8 ± 0.3 mnopqrs
Accordine×100×2021	494 ± 17 abcdefghij	81.0 ± 1.0 bcdefghijk	-28.8 ± 0.4 ghijk
Accordine×100×2022	472 ± 28 bcdefghijklm	67.8 ± 2.3 mnopqrstu	-26.9 ± 0.2 cd
Accordine×200×2020	447 ± 98 cdefghijklm	90.0 ± 1.6 ab	-29.8 ± 0.2 lmnopqrs
Accordine×200×2021	572 ± 22 abcd	81.4 ± 1.7 bcdefghijk	-28.9 ± 0.1 ghijk
Accordine×200×2022	464 ± 81 bcdefghijklm	65.8 ± 4.1 opqrstuv	-27.0 ± 0.3 cde
Nutans Afganistan×0×2020	562 ± 69 abcde	90.0 ± 6.0 ab	-29.5 ± 0.3 hijklmnopqrs
Nutans Afganistan×0×2021	488 ± 21 abcdefghijk	83.2 ± 2.0 bcdefghij	-28.7 ± 0.1 gh
Nutans Afganistan×0×2022	500 ± 36 abcdefghi	75.6 ± 6.0 defghijklmno	-26.0 ± 0.4 ab
Nutans Afganistan×100×2020	320 ± 46 m	91.8 ± 14.1 ab	-29.6 ± 0.3 klmnopqrs
Nutans Afganistan×100×2021	418 ± 23 defghijklm	81.2 ± 1.6 bcdefghijk	-28.7 ± 0.2 ghi
Nutans Afganistan×100×2022	442 ± 35 defghijklm	83.2 ± 2.8 bcdefghi	-25.7 ± 0.8 a
Nutans Afganistan×200×2020	339 ± 133 jklm	98.4 ± 8.5 a	-29.3 ± 0.4 ghijklmnopqr
Nutans Afganistan×200×2021	385 ± 15 hijklm	74.4 ± 1.5 efgijklmnopq	-28.6 ± 0.3 fg
Nutans Afganistan×200×2022	442 ± 55 defghijklm	84.2 ± 5.9 bcdefgh	-26.0 ± 0.5 ab
CPI 18197×0×2020	489 ± 82 abcdefghijk	77.2 ± 6.7 cdefghijklmn	-30.1 ± 0.1 s
CPI 18197×0×2021	405 ± 18 fghijklm	73.6 ± 2.5 fghijklmnopqr	-29.0 ± 0.2 ghijklm
CPI 18197×0×2022	532 ± 86 abcdefgh	72.4 ± 6.8 ijklnopqr	-26.7 ± 0.2 bc
CPI 18197×100×2020	412 ± 65 efgijklm	86.0 ± 9.8 abcde	-29.5 ± 0.3 ijklmnopqrs
CPI 18197×100×2021	422 ± 39 defghijklm	73.6 ± 1.5 fghijklmnopqr	-29.3 ± 0.2 ghijklmnopqr
CPI 18197×100×2022	447 ± 52 cdefghijklm	72.6 ± 1.5 hijklmnopqr	-26.6 ± 0.5 bc
CPI 18197×200×2020	337 ± 72 klm	83.6 ± 5.0 bcdefghi	-29.6 ± 0.3 jklmnopqrs
CPI 18197×200×2021	514 ± 30 abcdefgh	75.4 ± 1.7 defghijklmnop	-29.2 ± 0.2 ghijklmnopq
CPI 18197×200×2022	442 ± 53 defghijklm	70.6 ± 3.6 klmnopqrst	-26.7 ± 0.2 bc
CI 6388×0×2020	548 ± 136 abcdefg	87.6 ± 7.2 abcd	-29.9 ± 0.2 opqrs
CI 6388×0×2021	429 ± 18 defghijklm	80.0 ± 1.2 bcdefghijkl	-28.8 ± 0.3 ghijk
CI 6388×0×2022	504 ± 27 abcdefghi	73.4 ± 3.8 ghijklmnopqr	-27.0 ± 0.3 cde
CI 6388×100×2020	354 ± 33 ijkml	89.0 ± 10.7 abc	-30.0 ± 0.6 qrs
CI 6388×100×2021	445 ± 10 defghijklm	81.6 ± 1.1 bcdefghijk	-28.8 ± 0.3 ghij
CI 6388×100×2022	499 ± 39 abcdefghi	71.4 ± 2.6 jklmnopqrs	-27.4 ± 0.3 cde
CI 6388×200×2020	325 ± 127 lm	85.2 ± 4.4 abcdefg	-30.0 ± 0.4 pqrs
CI 6388×200×2021	508 ± 19 abcdefghi	81.0 ± 1.9 bcdefghijk	-28.7 ± 0.3 gh
CI 6388×200×2022	496 ± 59 abcdefghi	69.8 ± 5.7 lmnopqrst	-27.3 ± 0.4 cde

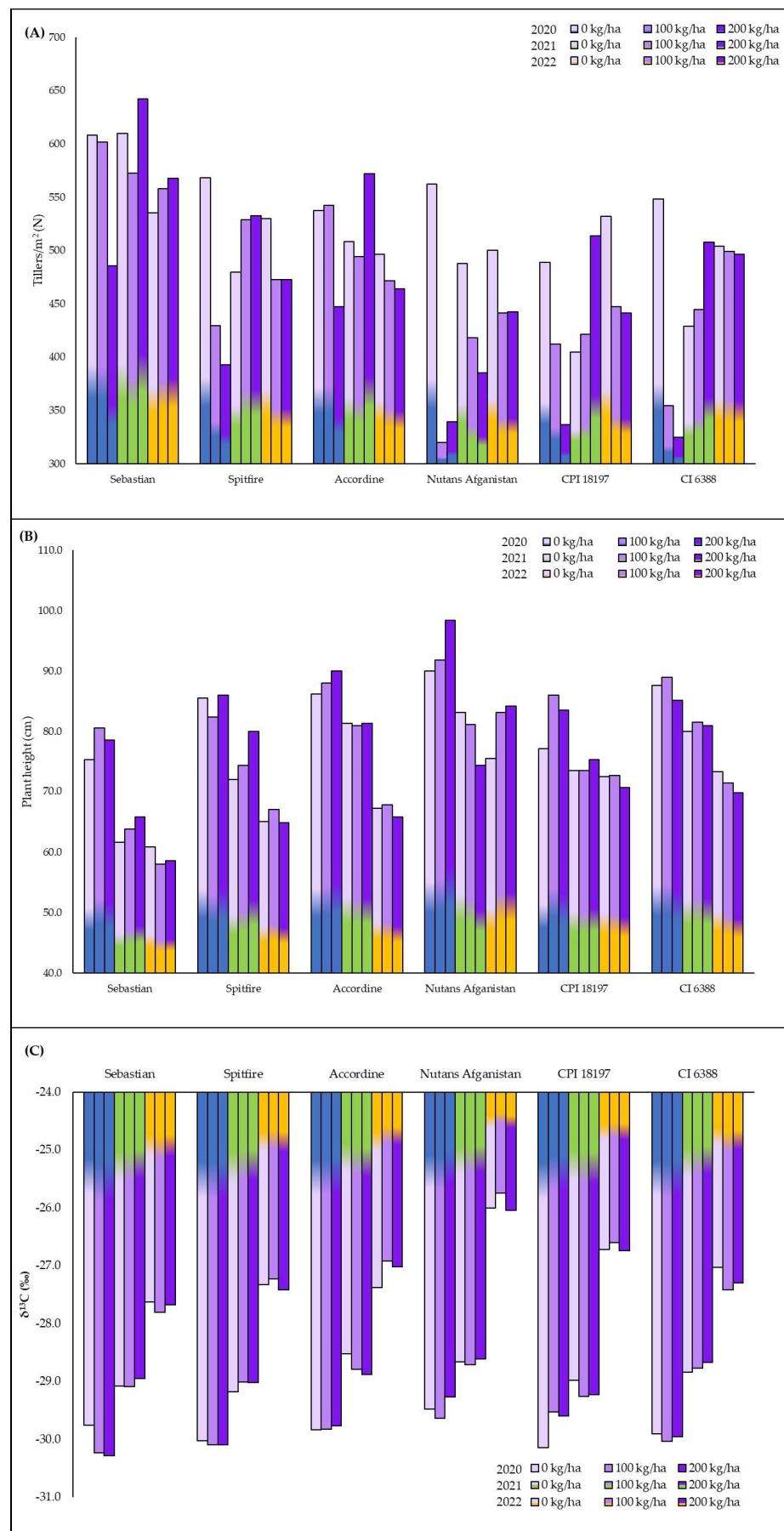


Figure S5. Interaction of genotype, fertilization, and season factors on the (A) tillering; (B) plant height; and (C) carbon isotope signature ($\delta^{13}\text{C}$). Fertilization doses 0, 100, or 200 kg of potassium per ha, season (2020–2022). Data represent means ($n = 5$). Statistical significance and SD values are shown in the Supplementary Table 4.

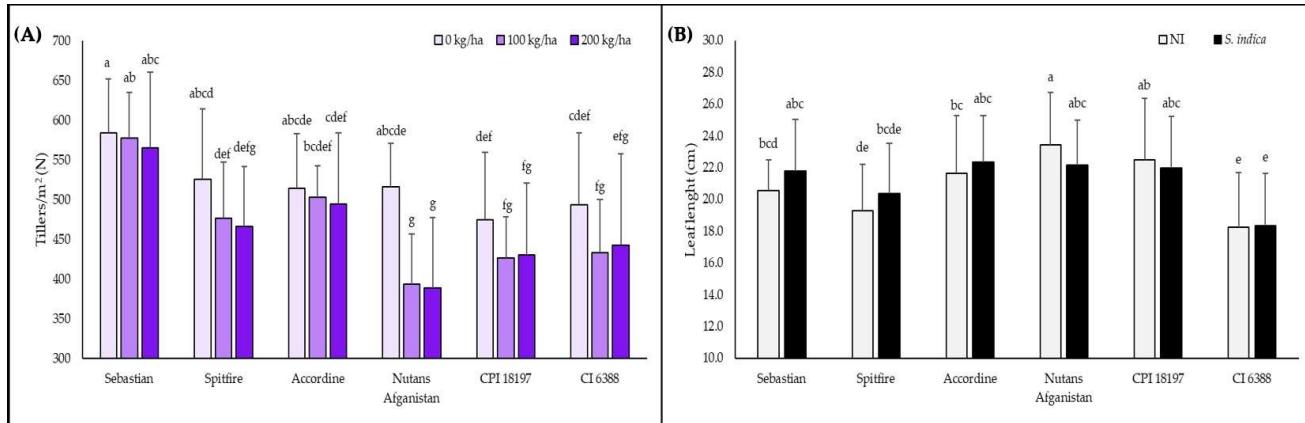


Figure S6. (A) Tillering of different genotypes in interaction with fertilization – columns represent means, bars SD ($n = 15$); (B) Leaf length in interaction of genotype and inoculation – columns represent means, bars SD ($n = 18$ for *S. indica*; $n = 27$ for NI); followed by the same letter if there was no statistical difference according to Tukey_{0.05} test.

Table S4. Interaction of genotype, fertilization and inoculation factors on the tillering. Treatment is in format genotype \times fertilization (0, 100, or 200 kg of potassium per ha) \times inoculation (NI – not inoculated, *S. indica* – *Serendipita indica*). Data represent means $\pm SD$ ($n = 6$ for *S. indica*; $n = 9$ for NI) followed by the same letter if there was no statistical difference according to Tukey_{0.05} test for unequal sample sizes.

Treatment	Tillers/m ² (N)
Sebastian \times 0 \times NI	580 \pm 63 abc
Sebastian \times 0 \times <i>S. indica</i>	591 \pm 80 abc
Sebastian \times 100 \times NI	561 \pm 36 abcd
Sebastian \times 100 \times <i>S. indica</i>	602 \pm 77 ab
Sebastian \times 200 \times NI	586 \pm 54 abc
Sebastian \times 200 \times <i>S. indica</i>	535 \pm 137 abcdefghijklm
Spitfire \times 0 \times NI	551 \pm 105 abcde
Spitfire \times 0 \times <i>S. indica</i>	488 \pm 34 abcdefghijklm
Spitfire \times 100 \times NI	477 \pm 89 abcdefghijklm
Spitfire \times 100 \times <i>S. indica</i>	478 \pm 32 abcdefghijklm
Spitfire \times 200 \times NI	452 \pm 73 defghijklm
Spitfire \times 200 \times <i>S. indica</i>	487 \pm 82 abcdefghijklm
Accordine \times 0 \times NI	540 \pm 67 abcdefg
Accordine \times 0 \times <i>S. indica</i>	475 \pm 56 abcdefghijklm
Accordine \times 100 \times NI	506 \pm 50 abcdefghijklm
Accordine \times 100 \times <i>S. indica</i>	499 \pm 23 abcdefghijklm
Accordine \times 200 \times NI	510 \pm 83 abcdefghijklm
Accordine \times 200 \times <i>S. indica</i>	470 \pm 101 abcdefghijklm
Nutans Afganistan \times 0 \times NI	522 \pm 68 abcdefghijklm
Nutans Afganistan \times 0 \times <i>S. indica</i>	509 \pm 28 abcdefghijklm
Nutans Afganistan \times 100 \times NI	402 \pm 57 hijklm

Nutans Afganistan×100× <i>S. indica</i>	380 ± 77 jk
Nutans Afganistan×200×NI	361 ± 102 k
Nutans Afganistan×200× <i>S. indica</i>	431 ± 42 defghijk
CPI 18197×0×NI	468 ± 83 abcdefghijk
CPI 18197×0× <i>S. indica</i>	486 ± 94 abcdefghijk
CPI 18197×100×NI	441 ± 56 defghijk
CPI 18197×100× <i>S. indica</i>	405 ± 39 ghijk
CPI 18197×200×NI	451 ± 92 defghijk
CPI 18197×200× <i>S. indica</i>	401 ± 87 hijk
CI 6388×0×NI	456 ± 53 bcdefghijk
CI 6388×0× <i>S. indica</i>	551 ± 108 abcdef
CI 6388×100×NI	430 ± 78 fghijk
CI 6388×100× <i>S. indica</i>	437 ± 56 defghijk
CI 6388×200×NI	461 ± 102 bcdefghijk
CI 6388×200× <i>S. indica</i>	416 ± 137 fghijk

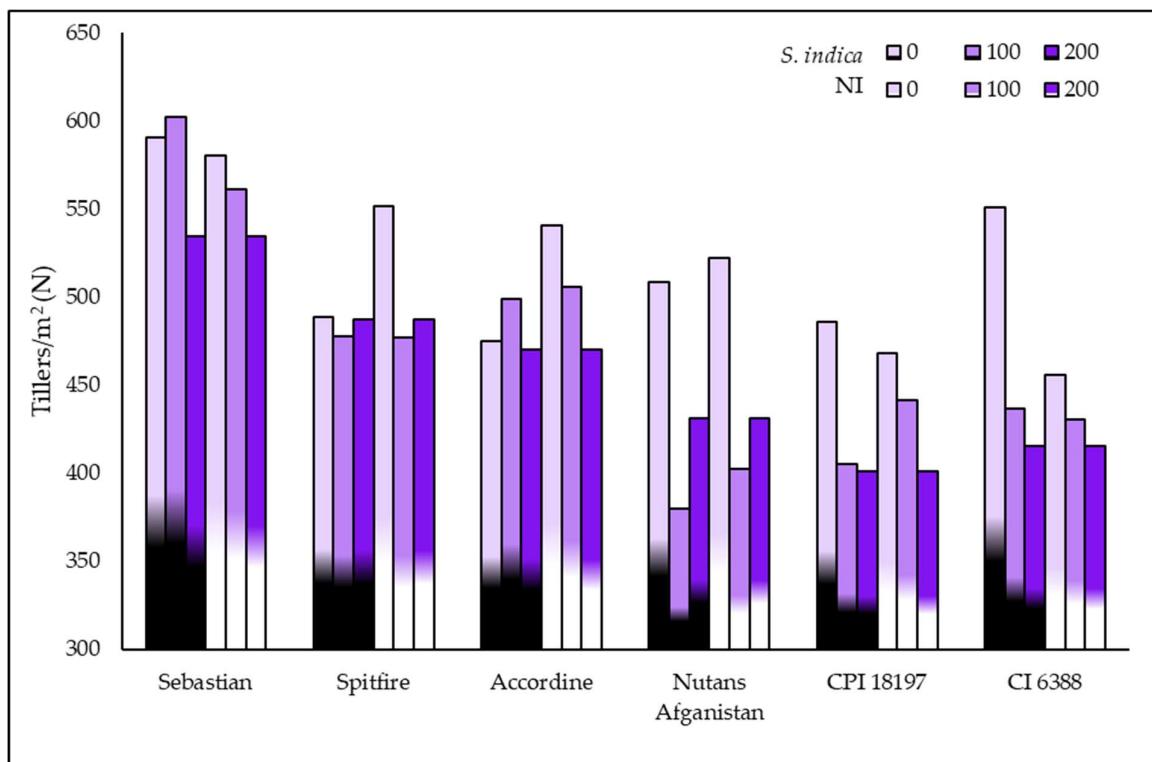


Figure S7. Interaction of genotype, fertilization, and inoculation factors on the tillering. Fertilization doses 0, 100, or 200 kg of potassium per ha, inoculation (NI – not inoculated, *S. indica* – *Serendipita indica*). Data represent means ($n = 6$ for *S. indica*; $n = 9$ for NI). Statistical significance and SD values are shown in the Supplementary Table 3.