

# **Leaf scorching following foliar fertilization of wheat with urea and urea-ammonium nitrate is caused by ammonium toxicity**

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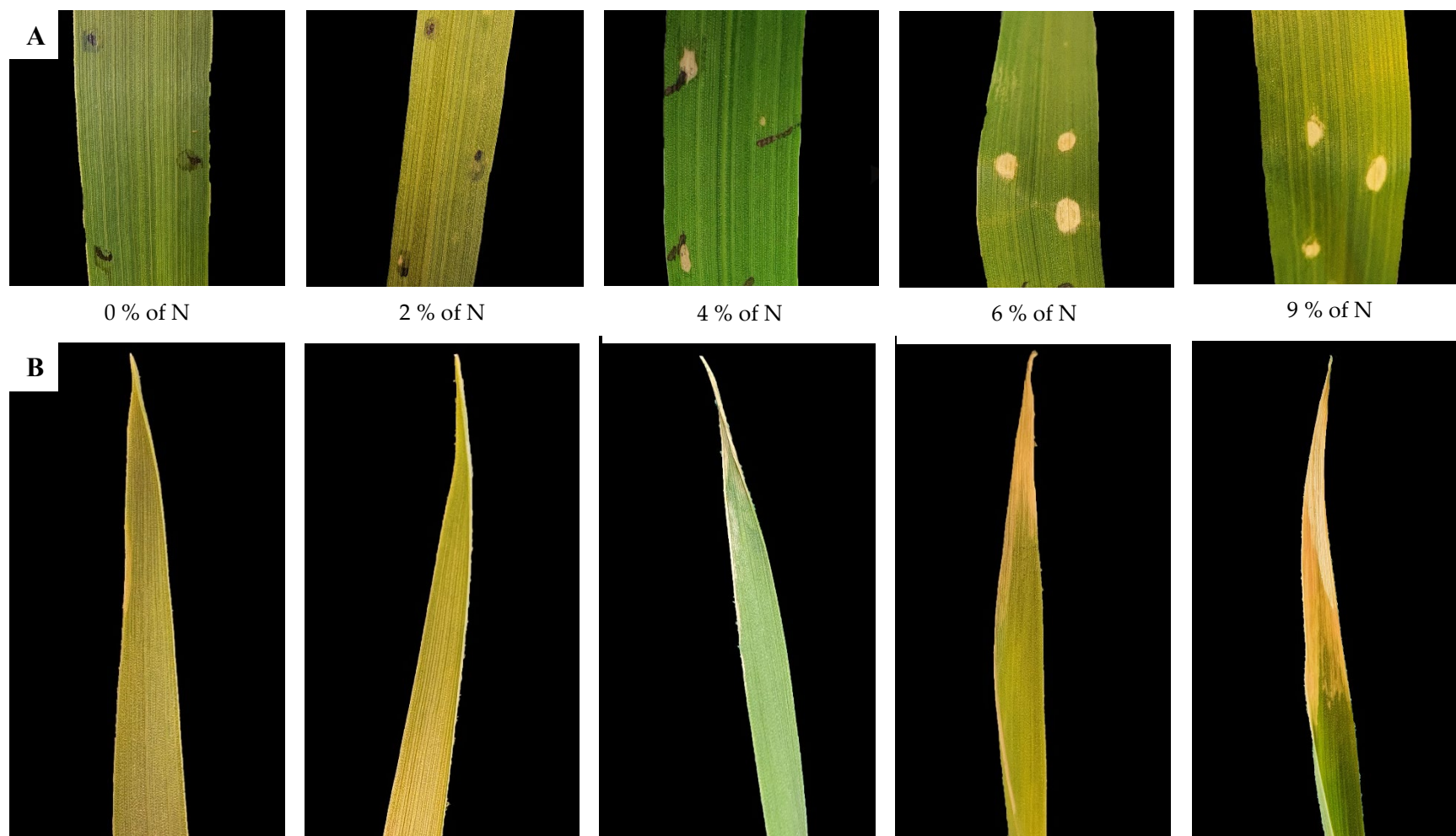
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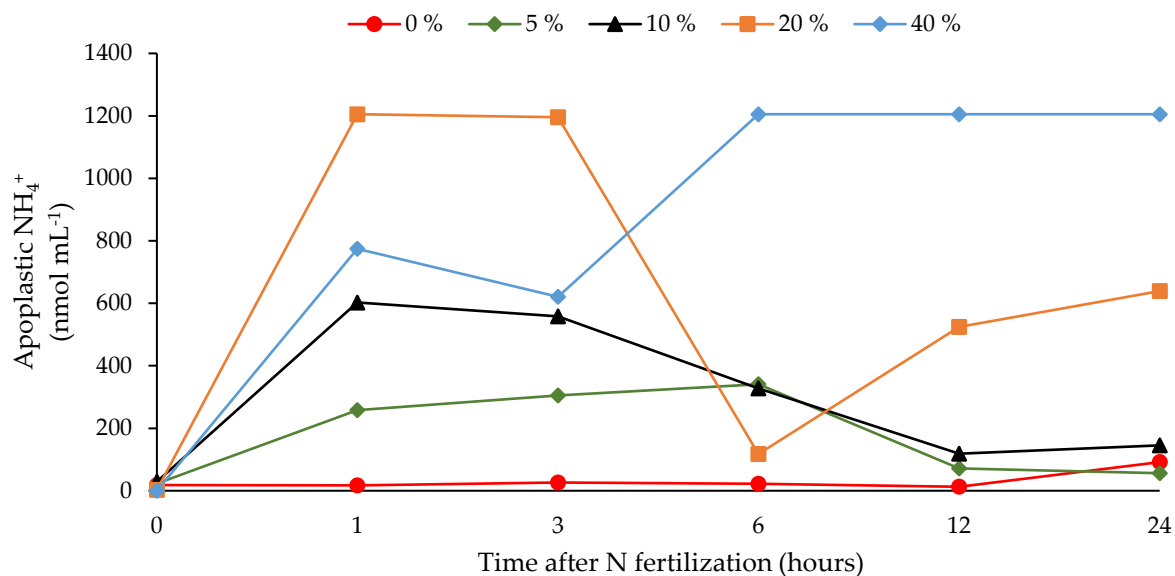
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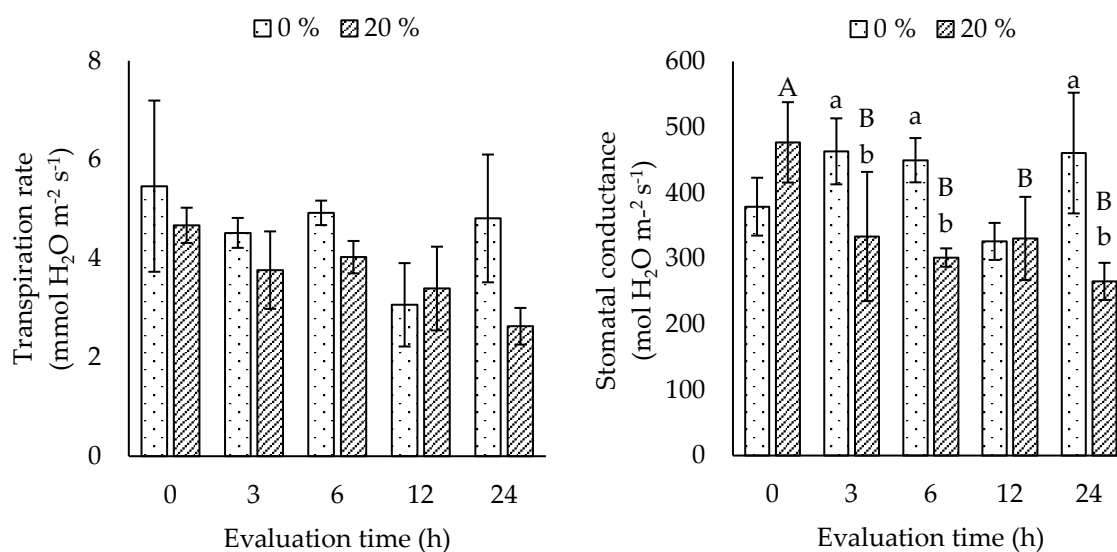
**Figure S1.** **A.** Spring wheat plant before foliar nitrogen fertilization (5-week-old), in the 2<sup>nd</sup> experiment; **B.** 3  $\mu$ L urea solution with surfactant droplets applied onto the middle section of the adaxial surface of the youngest fully expanded leaf (YFEL).



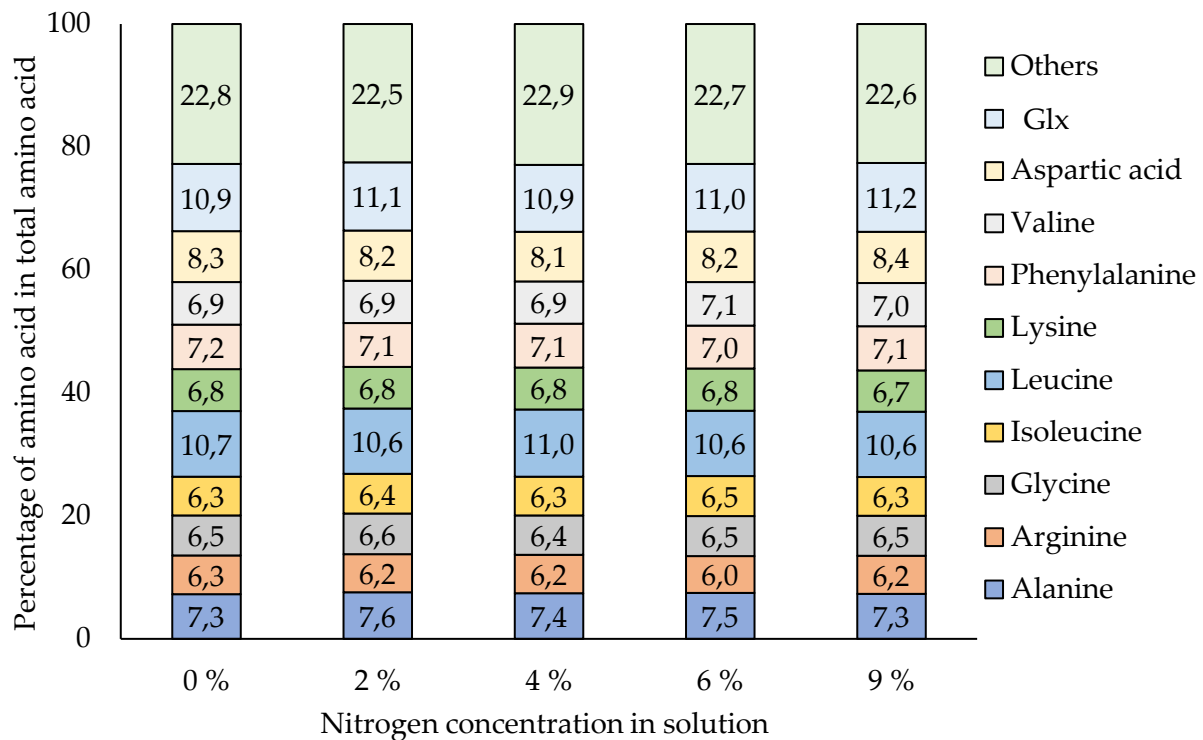
**Figure S2.** Nitrogen toxicity occurring 3 days after application of urea solution with surfactant onto the YFEL of wheat (cv. Harenda). Three droplets of each 3  $\mu$ l urea solution with a concentration of 0, 2, 4, 6 or 9 % N were pipetted onto the middle section of the adaxial surface of the YFEL. **A.** Scorching underneath the droplets; **B.** Scorching at the tip of the leaf.



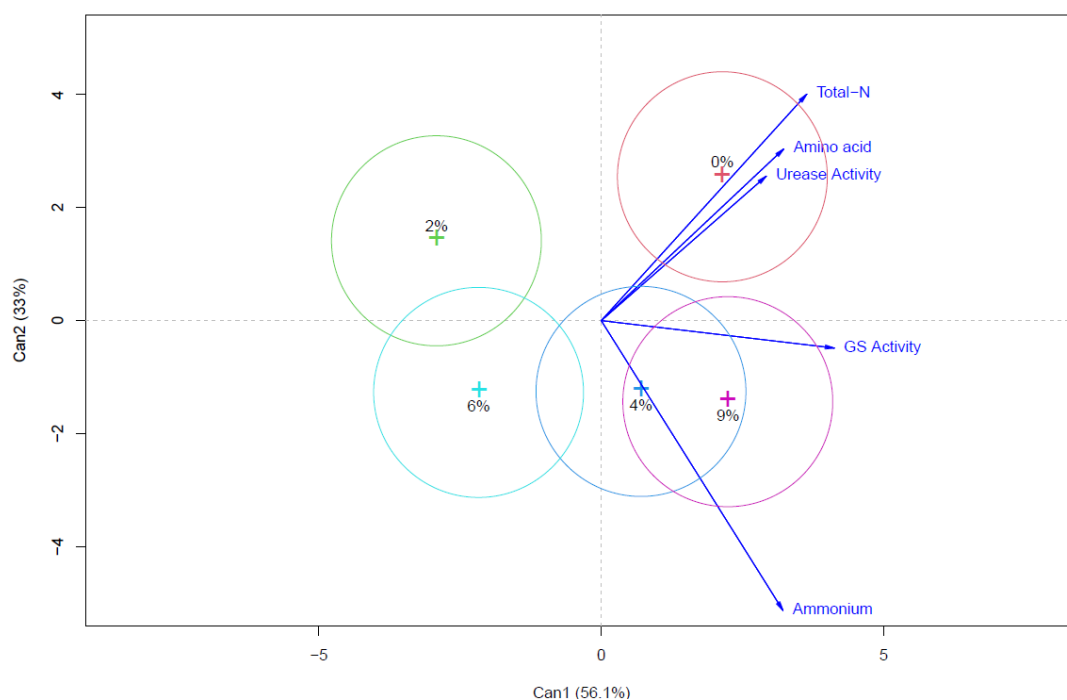
**Figure S3.** Apoplastic ammonium concentration ( $\text{NH}_4^+$ ) in the youngest and second youngest fully expanded leaves of wheat (cv. Amaretto) at 0, 1, 3, 6, 12, and 24 h after application of UAN solutions with increasing N concentration, viz. 0, 5, 10, 20, and 40 % N.



**Figure S4.** Transpiration rate and stomatal conductance of the youngest and second youngest fully expanded leaves of spring wheat (cv. Amaretto) after foliar fertilization with UAN solution (0 or 20 % N in solution) at 0, 3, 6, 12, and 24 h after foliar N-fertilization. Data are means of three independent biological replicates  $\pm$  standard error; small letters indicate difference between N treatments at each evaluation times, and capital letters indicate difference between evaluation times in each N treatment, according to Tukey's post-hoc test ( $p < 0.05$ ).



**Figure S5.** Amino acid concentrations in percentage of the total amino acid content in the YFEL of spring wheat (cv. Harenda) at 7 days after application of urea solution with surfactant onto the YFEL. Data are means of four independent biological replicates. "Glx" is the sum of Glutamine and Glutamate. "Others" are the sum of histidine, hydroxyproline, proline, serine, threonine, and tyrosine.



**Figure S6.** Canonical discriminant analysis of N parameters analyzed in the YFEL of spring wheat (cv. Harenda) foliar fertilized with urea with surfactant in different N concentrations at 7 days after foliar N-fertilization.

**Table S1.** Gas exchange parameters ( $C_i$  – internal  $CO_2$  concentration in stomatal chamber;  $g_s$  – stomatal conductance; VPD – vapor pressure deficit;  $A$  – photosynthesis rate;  $E$  – transpiration rate; and WUE – instantaneous water use efficiency) of the YFEL of spring wheat (cv. Harenda) at 7 days after application of urea solution with surfactant onto the YFEL.

N concentration	$C_i^{NS}$	$g_s^{NS}$	VPD <sup>NS</sup>	$A^{NS}$	$E^{NS}$	WUE
0 %	339.3	466.9	1.3	8.9	4.2	2.3 b
1 %	338.1	426.0	1.3	9.2	3.9	2.4 ab
3 %	337.1	401.9	1.3	9.3	3.8	2.5 ab
5 %	346.3	544.0	1.2	9.9	4.5	2.3 b
12 %	323.9	287.1	1.4	9.0	3.1	2.9 a
Mean	336.9	425.2	1.3	9.3	3.9	2.5
p-value	0.098	0.146	0.231	0.636	0.108	0.024
CV	4.7	46.4	12.7	15.2	25.2	18.0
MSD	22.7	283.4	0.2	2.0	1.4	0.6

Data are means of four independent biological replicates; letters indicate difference between N concentrations in solution according to Tukey's post-hoc test ( $p < 0.05$ ).

NS: no significant difference; CV: coefficient of variance; MSD: minimum significant difference.