

Phytotoxicity and Effect of Ionic Liquids on Antioxidant Parameters in Spring Barley Seedlings: The Impact of Exposure Time

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Table 1. Effect of ILs on the germination potential (GP) and germination rate (GR) of spring barley.

Concentration of ILs (mg·kg ⁻¹ of soil DM)	[BMIM][Br]		[TBA][Br]		[TBP][Br]	
	GP (%)	GR (%)	GP (%)	GR (%)	GP (%)	GR (%)
0	93.33 ± 4.08 ^a	95.00 ± 4.47 ^a	92.50 ± 5.24 ^a	99.17 ± 2.04 ^{ab}	97.50 ± 2.74 ^a	100.00 ± 0.00 ^a
1	86.67 ± 2.58 ^a	96.67 ± 4.08 ^a	92.50 ± 5.24 ^a	98.33 ± 2.58 ^{ab}	95.83 ± 4.92 ^a	99.12 ± 5.17 ^a
10	88.33 ± 5.16 ^a	95.00 ± 6.32 ^a	91.67 ± 4.08 ^a	97.50 ± 4.18 ^{ab}	99.17 ± 2.04 ^a	100.00 ± 0.00 ^a
100	85.83 ± 8.61 ^a	95.83 ± 3.76 ^a	95.00 ± 4.47 ^a	98.33 ± 4.08 ^{ab}	98.33 ± 2.58 ^a	100.00 ± 0.00 ^a
400	70.00 ± 14.83 ^b	94.17 ± 4.92 ^a	95.00 ± 4.47 ^a	99.17 ± 2.04 ^{ab}	93.33 ± 4.08 ^a	93.86 ± 3.96 ^b
700	63.33 ± 10.33 ^b	89.17 ± 8.61 ^a	89.17 ± 8.61 ^a	100.00 ± 0.00 ^a	93.33 ± 2.58 ^a	93.86 ± 6.15 ^b
1000	59.17 ± 8.61 ^b	93.33 ± 5.16 ^a	85.00 ± 8.37 ^a	93.33 ± 5.16 ^b	93.33 ± 4.08 ^a	93.86 ± 6.15 ^b

Data are means ± SD from 3 independent experiments. Values denoted by the same letters in the columns do not differ statistically at $p < 0.05$.

Table S2. Dry weight (g g⁻¹ FW) content in spring barley seedlings exposed to ILs.

Doses of ILs (mg kg ⁻¹ of soil DM)	Dry weight		
	[BMIM][Br]	[TBA][Br]	[TBP][Br]
Day 7			

0	0.0792 ± 0.0009 ^d	0.0861 ± 0.0031 ^{hi}	0.0789 ± 0.0011 ⁱ
1	0.0777 ± 0.0006 ^d	0.0838 ± 0.0018 ⁱ	0.0807 ± 0.0010 ^{hi}
10	0.0808 ± 0.0006 ^d	0.0867 ± 0.0006 ^{hi}	0.0814 ± 0.0012 ^{hi}
100	0.0909 ± 0.0044 ^c	0.0991 ± 0.0012 ^f	0.1088 ± 0.0007 ^f
400	0.1152 ± 0.0020 ^b	0.1192 ± 0.0004 ^{cde}	0.1169 ± 0.0020 ^e
700	0.1318 ± 0.0050 ^a	0.1191 ± 0.0010 ^{cde}	0.1261 ± 0.0040 ^d
1000	0.1315 ± 0.0024 ^a	0.1240 ± 0.0020 ^{cd}	0.1358 ± 0.0027 ^c
Day 14			
0	0.0783 ± 0.0022 ^d	0.0880 ± 0.0005 ^{hi}	0.0803 ± 0.0011 ^{hi}
1	0.0769 ± 0.0006 ^d	0.0888 ± 0.0014 ^{ghi}	0.0835 ± 0.0009 ^{hi}
10	0.0785 ± 0.0052 ^d	0.0875 ± 0.0002 ^{hi}	0.0866 ± 0.0020 ^h
100	0.0835 ± 0.0031 ^{cd}	0.0908 ± 0.0016 ^{ghi}	0.1049 ± 0.0019 ^{f,g}
400	0.1202 ± 0.0019 ^b	0.1169 ± 0.0053 ^{de}	0.1254 ± 0.0041 ^d
700	0.1307 ± 0.0028 ^a	0.1250 ± 0.0018 ^c	0.1397 ± 0.0022 ^c
1000	0.1318 ± 0.0007 ^a	0.1324 ± 0.0039 ^b	0.1491 ± 0.0030 ^b
Day 21			
0	0.0805 ± 0.0014 ^d	0.0877 ± 0.0036 ^{hi}	0.0810 ± 0.0013 ^{hi}
1	0.0798 ± 0.0012 ^d	0.0842 ± 0.0019 ^{hi}	0.0819 ± 0.0021 ^{hi}
10	0.0800 ± 0.0007 ^d	0.0914 ± 0.0028 ^{gh}	0.0828 ± 0.0023 ^{hi}
100	0.0822 ± 0.0017 ^d	0.0954 ± 0.0008 ^{f,g}	0.0994 ± 0.0011 ^g
400	0.1131 ± 0.0007 ^b	0.1167 ± 0.0008 ^e	0.1220 ± 0.0004 ^{de}
700	0.1313 ± 0.0041 ^a	0.1249 ± 0.0017 ^c	0.1529 ± 0.0008 ^b
1000	0.1322 ± 0.0007 ^a	0.1446 ± 0.0033 ^a	0.1692 ± 0.0030 ^a

Data are means ± SD (n=3); values denoted with the same letters form homogeneous groups at the level of p<0.05 (post-hoc Tukey's HSD test).

Table S3: Photosynthetic pigment (mg g⁻¹ DW) contents in spring barley seedlings exposed to [BMIM][Br].

Doses of [BMIM][Br] (mg kg ⁻¹ of soil DM)	Pigments			
	Chla	Chlb	Chla/Chlb	Chl(a + b)/Car
Day 7				
0	13.501 ± 0.072 ^{ab}	3.560 ± 0.014 ^a	3.814 ± 0.022 ^{efg}	5.208 ± 0.031 ^c
1	13.233 ± 0.165 ^{bc}	3.324 ± 0.064 ^b	3.982 ± 0.037 ^{cdef}	5.076 ± 0.076 ^{cde}
10	11.078 ± 0.076 ^e	2.955 ± 0.014 ^e	4.087 ± 0.045 ^{bcd}	4.928 ± 0.029 ^{efghi}
100	10.921 ± 0.015 ^g	2.521 ± 0.011 ^g	4.332 ± 0.015 ^{ab}	4.990 ± 0.018 ^{efgh}
400	10.832 ± 0.136 ^g	2.694 ± 0.052 ^f	4.021 ± 0.032 ^{cdef}	5.879 ± 0.035 ^a
700	8.035 ± 0.119 ⁱ	1.974 ± 0.049 ⁱ	4.175 ± 0.279 ^{bc}	5.692 ± 0.227 ^{ab}
1000	7.669 ± 0.057 ^j	1.772 ± 0.034 ⁱ	4.328 ± 0.051 ^{ab}	5.617 ± 0.024 ^b
Day 14				
0	13.379 ± 0.063 ^{bc}	3.346 ± 0.013 ^b	3.999 ± 0.016 ^{cdef}	4.855 ± 0.015 ^{fghi}
1	13.753 ± 0.040 ^a	3.161 ± 0.016 ^c	3.937 ± 0.114 ^{cdefg}	4.806 ± 0.032 ^{ghi}
10	13.083 ± 0.080 ^c	3.164 ± 0.016 ^c	4.135 ± 0.035 ^{abcd}	4.793 ± 0.011 ⁱ
100	12.522 ± 0.090 ^d	3.182 ± 0.071 ^c	4.352 ± 0.043 ^a	4.870 ± 0.010 ^{fghi}

400	10.771 ± 0.285^g	2.598 ± 0.071^{fg}	4.146 ± 0.015^{abc}	5.514 ± 0.013^b
700	7.945 ± 0.166^{ij}	1.729 ± 0.057^j	4.046 ± 0.135^{cde}	4.802 ± 0.066^{hi}
1000	6.936 ± 0.184^k	1.753 ± 0.028^l	3.957 ± 0.084^{cdefg}	5.017 ± 0.093^{def}
Day 21				
0	12.506 ± 0.055^d	3.314 ± 0.013^b	3.774 ± 0.007^{fg}	5.214 ± 0.006^c
1	11.793 ± 0.053^ef	3.088 ± 0.021^{cd}	3.820 ± 0.043^{fg}	4.992 ± 0.039^{fg}
10	11.529 ± 0.025^f	3.028 ± 0.029^{de}	3.895 ± 0.021^{defg}	4.976 ± 0.063^{ghi}
100	11.588 ± 0.023^f	3.115 ± 0.053^{cd}	3.721 ± 0.061^g	4.948 ± 0.059^{efghi}
400	8.425 ± 0.150^h	2.137 ± 0.037^h	3.943 ± 0.008^{cdefg}	5.090 ± 0.028^{cde}
700	6.993 ± 0.027^k	1.992 ± 0.038^i	4.182 ± 0.031^{abc}	5.064 ± 0.014^{cde}
1000	6.226 ± 0.139^l	1.501 ± 0.044^k	$4.150 \pm 0.^a$	5.195 ± 0.010^{cd}

Chla – chlorophyll *a*, Chlb – chlorophyll *b*, Chla/Chlb – chlorophyll *a*/chlorophyll *b*, Chl(*a* + *b*)/Car – (chlorophyll *a* + chlorophyll *b*)/carotenoids; Data are means \pm SD (n=3); values denoted with the same letters form homogeneous groups at the level of p<0.05 (post-hoc Tukey's HSD test).

Table S4: Photosynthetic pigment (mg g⁻¹ DW) contents in spring barley seedlings exposed to [TBA][Br].

Doses of [TBA][Br] (mg kg ⁻¹ of soil DM)	Pigments			
	Chla	Chlb	Chla/Chlb	Chl(<i>a</i> + <i>b</i>)/Car
Day 7				
0	13.704 ± 0.064^{bc}	3.538 ± 0.032^{abc}	3.874 ± 0.017^{abcde}	5.397 ± 0.024^{def}
1	14.597 ± 0.289^a	3.735 ± 0.115^a	3.909 ± 0.045^{abcd}	5.436 ± 0.020^{de}
10	14.009 ± 0.015^{ab}	3.603 ± 0.026^{ab}	3.889 ± 0.028^{abede}	5.470 ± 0.036^d
100	12.148 ± 0.325^e	3.160 ± 0.115^{cd}	3.845 ± 0.038^{abcdef}	5.685 ± 0.023^c
400	9.346 ± 0.170^h	2.438 ± 0.047^h	3.833 ± 0.013^{bedef}	6.208 ± 0.049^b
700	8.978 ± 0.065^h	2.425 ± 0.047^h	3.703 ± 0.052^f	6.366 ± 0.047^a
1000	7.939 ± 0.057^{ij}	2.096 ± 0.046^i	3.789 ± 0.059^{cdef}	6.152 ± 0.076^b
Day 14				
0	14.387 ± 0.308^{ab}	3.738 ± 0.079^a	3.848 ± 0.017^{abcdef}	5.192 ± 0.012^{jk}
1	13.256 ± 0.038^d	3.426 ± 0.043^{bc}	3.829 ± 0.040^{bcd}	5.264 ± 0.023^{ghij}
10	12.939 ± 0.111^{cd}	3.409 ± 0.072^{bcd}	3.797 ± 0.048^{bedef}	5.312 ± 0.047^{ghij}
100	11.194 ± 0.067^f	2.955 ± 0.027^{ef}	3.788 ± 0.013^{cdef}	5.198 ± 0.025^{ijk}
400	9.462 ± 0.076^h	2.521 ± 0.017^{gh}	3.753 ± 0.027^f	5.375 ± 0.051^{defg}
700	8.133 ± 0.202^i	2.159 ± 0.084^i	3.768 ± 0.059^{def}	5.383 ± 0.026^{defg}
1000	7.324 ± 0.154^i	1.917 ± 0.065^i	3.823 ± 0.095^{bedef}	5.332 ± 0.039^{efgh}
Day 21				
0	13.115 ± 0.420^{cd}	3.454 ± 0.130^{bc}	3.798 ± 0.038^{bedef}	5.456 ± 0.040^d
1	12.668 ± 0.556^{de}	3.331 ± 0.185^{cd}	3.804 ± 0.054^{bedef}	5.229 ± 0.011^{hijk}
10	10.400 ± 0.507^g	2.609 ± 0.149^{gh}	3.988 ± 0.071^a	4.910 ± 0.047^l
100	10.731 ± 0.143^{fg}	2.738 ± 0.043^{fg}	3.920 ± 0.009^{abc}	5.120 ± 0.019^k
400	9.256 ± 0.025^h	2.459 ± 0.013^h	3.765 ± 0.009^{def}	5.328 ± 0.042^{efgh}
700	7.918 ± 0.016^{ij}	2.010 ± 0.045^i	3.941 ± 0.098^{ab}	5.301 ± 0.057^{ghij}

1000	5.850 ± 0.056^k	1.486 ± 0.024^j	3.938 ± 0.042^{abc}	4.650 ± 0.020^m
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Chla – chlorophyll *a*, Chlb – chlorophyll *b*, Chla/Chlb – chlorophyll *a*/chlorophyll *b*, Chl(*a* + *b*)/Car – (chlorophyll *a* + chlorophyll *b*)/carotenoids; Data are means ± SD (n=3); values denoted with the same letters form homogeneous groups at the level of p<0.05 (post-hoc Tukey's HSD test).

Table S5: Photosynthetic pigment (mg g⁻¹ DW) contents in spring barley seedlings exposed to [TBP][Br].

Doses of [TBP][Br] (mg kg ⁻¹ of soil DM)	Pigments			
	Chla	Chlb	Chla/Chlb	Chl(<i>a</i> + <i>b</i>)/Car
Day 7				
0	12.712 ± 0.110^b	3.179 ± 0.015^{cd}	3.999 ± 0.032^{bcd}	4.922 ± 0.003^k
1	12.274 ± 0.008^{cd}	3.193 ± 0.013^c	3.845 ± 0.014^{fgh}	4.994 ± 0.012^j
10	12.428 ± 0.089^{bcd}	3.285 ± 0.041^{bc}	3.784 ± 0.021^{hi}	5.092 ± 0.012^i
100	10.742 ± 0.070^f	2.657 ± 0.030^f	4.043 ± 0.024^{bc}	5.359 ± 0.026^f
400	8.927 ± 0.070^i	2.370 ± 0.019^h	3.766 ± 0.007^i	6.075 ± 0.016^a
700	8.294 ± 0.068^j	2.075 ± 0.024^i	3.998 ± 0.021^{bcd}	5.797 ± 0.007^b
1000	7.852 ± 0.022^k	2.075 ± 0.021^i	3.784 ± 0.035^{hi}	5.842 ± 0.031^b
Day 14				
0	12.126 ± 0.260^d	3.382 ± 0.065^b	3.585 ± 0.013^j	5.356 ± 0.007^f
1	14.439 ± 0.226^a	3.996 ± 0.093^a	3.613 ± 0.028^j	5.458 ± 0.044^d
10	12.027 ± 0.050^d	3.059 ± 0.014^{de}	3.932 ± 0.031^{de}	5.039 ± 0.019^{ij}
100	10.228 ± 0.149^g	2.527 ± 0.060^g	4.073 ± 0.038^{ab}	5.276 ± 0.009^g
400	8.422 ± 0.092^j	2.142 ± 0.018^i	3.932 ± 0.012^{de}	5.373 ± 0.012^{ef}
700	7.224 ± 0.226^l	1.841 ± 0.058^j	3.924 ± 0.025^{de}	5.629 ± 0.021^c
1000	6.267 ± 0.046^m	1.548 ± 0.018^k	4.048 ± 0.017^{bc}	5.432 ± 0.042^{de}
Day 21				
0	11.312 ± 0.105^e	2.961 ± 0.043^e	3.820 ± 0.020^{fghi}	5.195 ± 0.011^h
1	12.596 ± 0.231^{bc}	3.320 ± 0.061^b	3.794 ± 0.004^{ghi}	5.240 ± 0.024^{gh}
10	9.603 ± 0.119^h	2.483 ± 0.044^{gh}	3.868 ± 0.028^{efg}	4.715 ± 0.024^{m}
100	9.673 ± 0.113^h	2.489 ± 0.037^{gh}	3.886 ± 0.014^{ef}	4.816 ± 0.019^l
400	7.179 ± 0.129^l	1.831 ± 0.028^j	3.921 ± 0.018^e	5.223 ± 0.020^{gh}
700	4.444 ± 0.070^n	1.167 ± 0.027^l	3.809 ± 0.041^{ghi}	4.771 ± 0.035^{lm}
1000	3.991 ± 0.070^o	0.968 ± 0.012^m	4.123 ± 0.021^a	4.563 ± 0.027^n

Chla – chlorophyll *a*, Chlb – chlorophyll *b*, Chla/Chlb – chlorophyll *a*/chlorophyll *b*, Chl(*a* + *b*)/Car – (chlorophyll *a* + chlorophyll *b*)/carotenoids; Data are means ± SD (n=3); values denoted with the same letters form homogeneous groups at the level of p<0.05 (post-hoc Tukey's HSD test).

Table S6: Malondialdehyde (MDA) (μg g⁻¹ FW) content in spring barley seedlings exposed to ILs.

Doses of ILs (mg kg ⁻¹ of soil DM)	Malondialdehyde (MDA) (μg g ⁻¹ FW)		
	[TBA][Br]	[BMIM][Br]	[TBP][Br]

Day 7				
0	35.937 ± 1.205 ^{ab}	29.509 ± 1.545 ^{de}	31.177 ± 0.369 ^{bc}	
1	35.381 ± 0.347 ^b	26.700 ± 1.437 ^{fg}	27.999 ± 0.683 ^e	
10	26.858 ± 0.586 ^{cde}	29.614 ± 0.653 ^{cde}	29.113 ± 0.136 ^{de}	
100	35.069 ± 2.173 ^b	27.565 ± 1.101 ^{efg}	32.777 ± 0.689 ^{ab}	
400	38.675 ± 0.233 ^a	32.513 ± 0.896 ^b	30.595 ± 0.068 ^{cde}	
700	36.888 ± 0.283 ^{ab}	31.976 ± 0.606 ^{bed}	31.171 ± 0.291 ^{bc}	
1000	35.179 ± 0.669 ^b	28.658 ± 1.167 ^{ef}	33.014 ± 0.493 ^a	
Day 14				
0	22.111 ± 0.403 ^{fgh}	27.380 ± 0.877 ^{efg}	23.657 ± 0.934 ^f	
1	26.008 ± 0.878 ^{cde}	23.251 ± 1.584 ^{hi}	19.923 ± 0.838 ^h	
10	23.781 ± 1.728 ^{efg}	25.769 ± 0.803 ^{gh}	21.591 ± 0.239 ^g	
100	28.546 ± 0.892 ^c	29.471 ± 0.472 ^{def}	22.468 ± 0.192 ^{fg}	
400	27.794 ± 0.405 ^c	35.569 ± 1.312 ^a	28.275 ± 0.359 ^e	
700	21.553 ± 0.229 ^{gh}	35.388 ± 0.395 ^{ab}	29.407 ± 0.651 ^{de}	
1000	20.607 ± 0.607 ^h	32.293 ± 0.941 ^{bc}	28.795 ± 0.517 ^e	
Day 21				
0	17.242 ± 0.818 ^{ij}	20.022 ± 0.178 ^{jk}	19.718 ± 0.413 ^{hi}	
1	14.745 ± 0.298 ⁱ	17.133 ± 0.376 ^l	18.191 ± 0.567 ⁱ	
10	15.207 ± 0.360 ^{ij}	16.223 ± 0.454 ^l	18.325 ± 0.354 ^{hi}	
100	20.050 ± 0.295 ^{hi}	18.519 ± 0.118 ^{kl}	23.295 ± 0.295 ^f	
400	24.651 ± 0.559 ^{def}	20.840 ± 0.627 ^{ijk}	22.878 ± 0.310 ^{fg}	
700	20.185 ± 0.851 ^{hi}	22.306 ± 0.326 ^{ij}	27.889 ± 0.411 ^e	
1000	21.020 ± 0.594 ^{gh}	21.116 ± 0.632 ^{ijk}	23.952 ± 0.965 ^f	

Data are means ± SD (n=3); values denoted with the same letters form homogeneous groups at the level of p<0.05 (post-hoc Tukey's HSD test).

Table S7. Superoxide dismutase (SOD) (U mg⁻¹ protein) activity in spring barley seedlings exposed to ILs.

Doses of ILs (mg kg ⁻¹ of soil DM)	Superoxide dismutase (SOD) (U mg ⁻¹ protein)		
	[TBA][Br]	[BMIM][Br]	[TBP][Br]
Day 7			
0	12.455 ± 0.818 ^a	11.329 ± 0.774 ^a	8.067 ± 0.210 ^{bcd}
1	11.179 ± 0.773 ^{ab}	11.342 ± 0.698 ^a	8.659 ± 1.002 ^{bed}
10	9.795 ± 0.709 ^{abc}	10.343 ± 0.330 ^{ab}	6.447 ± 0.882 ^e
100	9.630 ± 0.296 ^{bcd}	10.228 ± 0.276 ^{abc}	6.696 ± 0.462 ^{de}
400	8.470 ± 0.725 ^{cde}	10.422 ± 0.800 ^{ab}	6.844 ± 0.264 ^{de}
700	9.050 ± 0.561 ^{cde}	10.226 ± 0.474 ^{abc}	7.293 ± 0.714 ^{cde}
1000	8.232 ± 0.074 ^{cde}	10.367 ± 0.642 ^{ab}	7.218 ± 0.162 ^{cde}
Day 14			
0	8.499 ± 0.077 ^{cde}	8.050 ± 0.320 ^{cd}	8.757 ± 0.440 ^{bcd}
1	8.481 ± 0.534 ^{cde}	7.410 ± 1.458 ^d	9.096 ± 0.992 ^{bc}
10	7.700 ± 0.332 ^e	7.648 ± 0.951 ^d	8.205 ± 0.256 ^{bcd}

100	7.694 ± 0.321^e	6.686 ± 0.514^d	7.878 ± 0.385^{bcde}
400	7.541 ± 0.498^e	6.783 ± 0.293^d	8.171 ± 0.270^{bcde}
700	7.809 ± 0.147^e	8.424 ± 0.976^{bcd}	8.099 ± 0.215^{bcde}
1000	7.478 ± 0.440^e	8.581 ± 0.723^{bcd}	8.973 ± 0.234^{bc}
Day 21			
0	8.410 ± 0.263^{cde}	7.105 ± 0.433^d	8.972 ± 0.844^{bc}
1	8.016 ± 0.112^{cde}	7.401 ± 1.088^d	9.581 ± 1.389^{ab}
10	9.782 ± 1.522^{abcd}	6.430 ± 0.437^d	8.639 ± 0.170^{bcd}
100	8.210 ± 0.346^{cde}	6.514 ± 0.136^d	8.208 ± 0.560^{bcde}
400	7.678 ± 0.173^e	6.530 ± 0.340^d	8.955 ± 1.311^{bc}
700	9.168 ± 0.782^{cde}	6.404 ± 0.733^d	9.733 ± 0.363^{ab}
1000	9.653 ± 0.182^{bcd}	6.386 ± 0.114^d	11.350 ± 0.473^a

Data are means \pm SD (n=3); values denoted with the same letters form homogeneous groups at the level of p<0.05 (post-hoc Tukey's HSD test).