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Sensitivity of Barley Varieties to Aluminum Ions: Separately Effects and Combine with Iron Ions

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ABSTRACT

Differences in the barley varieties have been revealed from tolerance to iron (Fe) and aluminum (Al) ions as well as to their combined effect. Received results allowed to separate barley variety into some (three) groups: the first—Al-tolerant varieties, the second—Al-sensitive ones and third—moderately resistant variety. The increased concentration of Fe had practically no effect on biometric (seed germination energy) and cytogenetics (frequency of chromosome aberrations and mitotic index) parameters as compared to the reference values. At the same time, iron ion significantly reduces the phytotoxic effect for Al-tolerant varieties in case of these elements jointly presented in solution.

Keywords: Barley Varieties; Aluminum and Iron Ions; Phytotoxic Effect

1. Introduction

The adverse Al impact on plants has long been known and free aluminum reduces crop productivity on acid soils [1-3]. The tolerance to aluminum in plants is found to be dependent on the properties of plant varieties [4,5]. However, there is no unified approach to determining plant resistance to changes in acidity and the planning of experiments, and the following analysis of results are being performed disregarding the available inter-variety differences relative to ionic toxicity which is more pronounced than differences in varieties in some cases [6,7].

As the acid load on soil increases, in its upper horizons what accumulated is also free iron [8] stipulating the combined ionic impact of these elements on different components of cenosis. In this case, an increased concentration of Al and Fe ions in the uptake root area induces differently oriented responses in various varieties of plants [3,9]. The development mechanism is not clear.

The main aim of the paper is to establish the barley varieties which are sensitive or tolerant to aluminium and iron as well as to their combined effect.

2. Materials and Methods

2.1. Treatment of Barley Seeds

Fourteen varieties of barley (*Hordeum vulgare* L.) were selected for the experiment: Turyngil, Gorinsky, Nur, Bios, Ataman, Auriga, Getman, Binom, Ratnik, Margret, Gonar, Perun, Zevs and Sellar.

To assess the separate and combined impact of metals, the seeds of barley varieties (100 in recurrence) in petri dishes with the salt solutions of iron and aluminium ($FeCl_3$ a $6H_2O$ and $AlCl_3$ a $6H_2O$) were placed in a thermostat and left for 3 days in dark at 25˚C to germinate. Each experiment was repeated five times. Ion concentration of metals in a solution at pH 5 was chosen to be equal to that in soil at pH < 5, *i.e.* 0.3 mg/l and 0.5 mg/l for Fe^{3+} and Al^{3+}, respectively [2].

2.2. Morphometric and Cytogenetic Measurements

The effect of Fe and Al chlorides on barley seeds was assessed from morphometric (seed germination energy) and cytogenetic (mitotic index MI is the fraction of cells being in mitosis to the total number of cells in a root merisystem; frequency of chromosome aberrations) indices [10,11]. Changes in cytogenetic indices were estimated after two-day seed germination in the salt solutions of metals for roots (5 - 10 mm long) fixed in acetoacetic alcohol. After colouring with acetocarmine, the crushed specimens were analyzed with the help of a microscope having a 400-fold magnification. Seeds germinated in distilled water were used as reference ones.

Standard techniques of variational statistics with the software MathCAD 2001iprofessional and Microsoft Office Excel 2003 have been used to process experimental data. Reliability of differences was estimated by Student's t-test. The degree of difference $P < 0.05$ was accepted as statistically significant in estimating results.

3. Results

3.1. Seed Germination Energy

The results of morphometric indices analyzed for various varieties of barley seeds have shown that the chosen concentration of Fe ion had a minor effect on deviations of these indices from the reference ones (**Table 1**).

Variations in the seed germination energy in two-day barley seedlings have been observed under the action of aluminum (separately and with iron). For the varieties Bios, Ratnik and Zevs in case of barley germination in aluminium solution the germination energy index accounted for 16.6% on the average, *i.e.* 4.8 fold lower than the reference value (**Table 1**). For the varieties Gorinsky, Getman, Margret and Gonar the index variations were insignificant, *i.e.* the germination energy, on the average, was 1.6 fold lower than the reference.

It should be noted that although certain deviations in the germination energy indices from their reference values have been identified for some varieties (Turyngil, Nur, Ataman, Auriga, Binom, Perun and Sellar), they were not so significant as compared to the varieties Bios, Ratnik and Zevs.

3.2. Cytogenetic Parameters

The results of MI and the frequency of chromosome aberrations have shown that the chosen concentration of Fe ion had a minor effect on deviations of these indices from the reference ones as well as morphometric measurements (**Table 2**).

For the varieties Bios, Ratnik and Zevs in case of barley treatment in aluminium solution the MI amounted to 2.9%, *i.e.* 2.9 fold lower than the reference. For the varieties Gorinsky, Getman, and Gonar MI had decreased of 1.2 to 1.6 times.

Deviations in MI parameters from their reference values for some cultivars (Turyngil, Nur, Ataman, Auriga, Binom, Perun and Sellar) were not so significant as compared to the varieties Bios, Ratnik and Zevs but more than group varieties Gorinsky, Getman, and Gonar.

The level of cell chromosome aberrations in the barley root merisystem in the presence of Fe ion has reduced the total number of aberrant cells stipulated by aluminum effects (**Table 3**).

The frequency of chromosome aberrations under the action of $AlCl_3$ in the barley varieties Bios, Ratnik and

Table 1. Germination energy (%) of barley varieties.

Barley cultivars	Variants			
	Reference	$FeCl_3$	$AlCl_3$	$FeCl_3 + AlCl_3$
Bios	78.2 + 3.6	83.8 + 5.2	16.8 + 3.5a	48.2 + 4.3b
Ratnik	78.8 + 7.2	88.0 + 6.3	17.0 + 5.8a	32.8 + 5.6b
Zevs	85.2 + 8.4	92.0 + 6.4	16.0 + 5.0a	47.4 + 12.0b
Gorinsky	90.4 + 3.3	87.8 + 11.2	76.2 + 3.2a	80.0 + 3.3
Margret	89.2 + 7.7	91.4 + 7.5	77.2 + 4.2a	80.8 + 2.2
Gonar	89.2 + 6.7	90.2 + 6.4	72.4 + 3.3a	82.0 + 4.2b
Getman	84.6 + 4.7	90.2 + 5.2	68.8 + 3.5a	73.4 + 3.8
Binom	90.6 + 12.7	90.0 + 3.3	41.8 + 16.1a	73.8 + 9.7b
Ataman	86.6 + 4.1	89.0 + 2.2	45.2 + 8.0a	78.0 + 3.6b
Auriga	87.2 + 7.5	93.8 + 4.4	49.0 + 4.7a	68.2 + 3.3b
Turyngil	76.8 + 3.2	82.1 + 5.33	50.8 + 2.5a	61.8 + 2.8b
Perun	91.4 + 6.9	95.0 + 5.9	41.6 + 8.3a	69.4 + 4.6b
Nur	85.2 + 6.4	87.4 + 6.4	49.4 + 5.0a	75.6 + 5.8b
Sellar	86.4 + 8.3	91.0 + 7.7	45.0 + 6.3a	69.2 + 5.0b

From now on the differences in tables are significantly at $P < 0.05$: a—relative to reference values; b—relative to $AlCl_3$ variant.

Table 2. Mitotic index of barley seedlings.

Barley caltivars	Mitotic index (MI), %			
	Reference	$FeCl_3$	$AlCl_3$	$FeCl_3 + AlCl_3$
Bios	8.4 + 0.6	8.9 + 0.7	3.0 + 0.3a	5.4 + 1.0b
Ratnik	8.1 + 0.6	8.0 + 0.2	3.2 + 0.8a	5.1 + 0.2b
Zevs	8.9 + 0.3	8.3 + 0.9	2.7 + 0.5a	6.1 + 0.6b
Gorinsky	9.1 + 0.7	9.4 + 0.7	7.8 + 0.6a	8.1 + 0.4
Margret	8.4 + 1.1	9.3 + 0.8	8.2 + 0.7a	8.5 + 0.8
Gonar	9.1 + 0.2	8.7 + 0.5	6.2 + 0.3a	6.8 + 0.5
Getman	10.0 + 0.3	9.3 + 0.8	6.1 + 0.2a	7.2 + 0.3b
Binom	9.7 + 1.1	9.9 + 0.8	4.6 + 0.8a	6.7 + 0.6b
Ataman	9.5 + 0.6	9.1 + 0.6	5.7 + 0.8a	8.2 + 0.3b
Auriga	9.6 + 0.5	9.7 + 0.6	5.8 + 0.2a	8.1 + 0.2b
Turyngil	9.3 + 0.7	8.3 + 0.6	6.5 + 0.4a	7.7 + 0.4b
Perun	9.3 + 0.7	9.1 + 1.2	4.6 + 0.8a	7 + 0.7b
Nur	9.8 + 1.1	10.0 + 0.9	5.5 + 0.5a	7.6 + 0.7b
Sellar	9.3 + 1.1	8.8 + 1.5	4.6 + 0.8a	6.9 + 0.7b

Table 3. Frequency of aberrant cells, %.

Barley cultivars	Variants			
	Reference	$FeCl_3$	$AlCl_3$	$FeCl_3 + AlCl_3$
Bios	1.7 + 0.3	1.5 + 0.4	6.8 + 0.4a	4.1 + 0.7b
Ratnik	1.8 + 0.3	1.9 + 0.2	7.3 + 0.5a	5.7 + 0.6b
Zevs	1.4 + 0.6	0.9 + 0.2	7.4 + 0.5a	3.2 + 0.4b
Gorinsky	0.7 + 0.3	0.9 + 0.2	1.8 + 0.3a	1.6 + 0.7
Margret	1 + 0.3	1.1 + 0.2	2.3 + 0.6a	2.5 + 0.5
Gonar	1.2 + 0.2	1.1 + 0.4	2.9 + 0.1a	2.6 + 0.1
Getman	0.7 + 0.4	0.7 + 0.5	3.4 + 0.3a	2.8 + 0.4
Binom	1.4 + 0.4	1.3 + 0.5	4.7 + 0.6a	3.1 + 0.3b
Nur	1.6 + 0.3	1.3 + 0.8	3.8 + 0.5a	2.2 + 0.3b
Auriga	1.6 + 0.6	1.5 + 0.4	4.6 + 0.8a	2.5 + 0.5b
Turyngil	2.6 + 0.2	2.8 + 0.1	5.2 + 0.2a	4.3 + 0.3b
Perun	1.2 + 0.8	1.3 + 0.5	4.6 + 0.6a	3.2 + 0.6b
Ataman	1.5 + 0.5	1.4 + 0.3	4.8 + 0.6a	2.1 + 0.7b
Sellar	1.3 + 0.6	1.1 + 0.3	4.8 + 0.8a	3.2 + 0.7b

Zevs was more than twice as large as for another ones (**Table 3**). However, in a combined impact of $FeCl_3$ and $AlCl_3$ the frequency of aberrant cells has decreased and exceeded the reference value (Bios, Ratnik, Zevs). It should be noted that iron practically does not reduce the toxic effect of aluminum in the criterion of mitotic index suppression for the Al-tolerant cultivars Gorinsky, Margret and Gonar.

Table 4. Index deviation (%) from references.

Barley cultivars	Germination energy		Mitotic index		Deviation sum	
	$AlCl_3$	$FeCl_3 + AlCl_3$	$AlCl_3$	$FeCl_3 + AlCl_3$	$AlCl_3$	$FeCl_3 + AlCl_3$
Sensitive						
Bios	−78.6	−38.8	−64.2	−35.7	−142.8	−74.5
Ratnik	−78.4	−38.9	−60.4	−37.0	−138.8	−75.8
Zevs	−81.2	−44.2	−69.6	−31.4	−150.8	−75.6
Tolerant						
Gorinsky	−15.7	−11.5	−14.2	−10.9	−29.9	−22.4
Margret	−13.4	−9.4	−2.3	1.2	−15.7	−8.2
Gonar	−18.8	−8.2	−31.8	−25.2	−50.6	−33.4
Getman	−18.6	−13.2	−39.0	−28.0	−57.6	−41.2
Intermediate						
Binom	−53.8	−18.5	−52.5	−31.0	−106.3	−49.5
Ataman	−47.8	−9.9	−40.0	−13.6	−87.8	−23.5
Auriga	−43.8	−21.7	−39.5	−15.6	−83.3	−37.3
Turyngil	−33.8	−19.5	−30.1	−17.2	−63.9	−36.7
Perun	−54.4	−24.0	−50.5	−24.7	−104.9	−48.7
Nur	−42.0	−11.2	−43.8	−22.4	−98.5	−45.7
Sellar	−48.0	−19.9	−50.5	−25.8	−85.8	−33.6

(-)—decrease relative to reference values; deviations for $FeCl_3$ ranged within (−10.7)% - (+11.6)%.

4. Discussion

The results analysed allowed all the barley varieties to be conventionally divided into three groups: Al-tolerant cultivars, *i.e.* Gorinsly, Getman, Margret, Gonar with the total deviation for two indices (germination energy and MI) less than 60%; Al-sensitive ones, *i.e.* Bios, Ratnik and Zevs, the total deviation of which exceeds 130 % and moderately resistant cultivars, *i.e.* Ataman, Turyngil, Nur, Auriga, Binom, Perun and Sellar with the total value of 60% to 110% (**Table 4**).

Al and Fe ions available in a solution for seed germination seem to favor the suppression of toxic aluminum effects, especially for Al-sensitive cultivars and increased the germination energy index as compared to seed germination in aluminum solution only (by 2.6 times for these varieties, on the average).

The mechanism of protective Fe effects is complicated and often related to both the biochemical variations proceeding at a cellular level (e.g. synthesis of proteins-phytochelates) and the observed induction of other toxicant-protective mechanisms in plant organisms. The observed differences in response of barley cultivars to Al and Fe seem to be stipulated by genetically determined mechanisms of plant tolerance [3,7]. In addition, the impact of Fe ion on seedlings of tolerant barley cultivars was not followed by weakening of Al effect. For Al-tolerant cultivars of barley its content in root protoplasts may be low, remaining rather high in root tissues as a whole. There is a clear evidence of Al absorption, *i.e.* its diffusion via plasmalemma [12]. Once in a cell, aluminum is localized mostly in the nucleus and its content in the cell nuclea of sensitive cultivars is 2 - 3 fold as large as in the cells of tolerant cultivars. The binding of Al with nucleic acids, mainly DNA, causes serious disturbances in functioning of growing root cells. Al tolerance in barley has been found to be controlled by a dominant gene [13]. In our opinion this fact is again indicative of available adaptable mechanisms capable for reducing the effect of toxic environmental factors on plant organisms.

5. Conclusions

Our findings concerning the effect of Al and Fe on variations in the biometric and cytogenetic indices of seedlings in various barley cultivars allow one to conclude:

- Fourteen barley cultivars selected for studies differ in their tolerance.
- In a degree of index deviations (seed germination energy and MI) from reference values, these barley cultivars could be conventionally divided into 3 groups: highly Al-sensitive, tolerant and moderately resistant.
- Mechanisms of Al tolerance in barley seedlings are complex and genetically controlled. The frequency of chromosome aberrations is 4 - 5 fold and only 2.5 - 5 fold as large as the level of spontaneous mutations for sensitive and tolerant cultivars, respectively.
- The increased concentration of Fe in a solution for germinating various cultivars of barley has practically no effect on variations in the germination energy and MI of seedlings as compared to the reference values; at the same time the joint presence of Fe and Al reduces the toxic aluminum effects that are especially pronounced in Al-sensitive cultivars.

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