ALUMINIUM TOLERANCE OF BARLEY
I. EFFICIENCY OF *IN VIVO* PROCEDURES IN ESTIMATION OF GENOTYPIC DIFFERENCES

Petre Maxim and Zoe Duță

ABSTRACT

A number of 24 genotypes of six- and two-rowed barley, released in Romania, Germany and USA, were tested for their tolerance to Al using four *in vivo* procedures. Tolerance was measured by the inhibitory effect of Al ions and low pH values on root development in five-day old seedlings. Dayton, Sunrise and Smooth Awn, with relative highier root elongation rate (71%) were considered as having the maximum tolerance to Al ions. Sensitive genotypes registered maximum values for hematoxylin stainability at 0.09 mM Al^{3+}. A potential redox (NADH / ferrycianide) was evidentiated at the root level. Mitotic index was significantly lower after four hours of treatment with AL in sensitive genotypes.

Key words: *Hordeum vulgare, Hordeum distichum,* Al toxicity

INTRODUCTION

Growth, development and yielding ability of agricultural crops on acid soils are strongly affected by the omnipresence of metal ions, whose solubility is largely increased by the acid pH of the rizosphere.

Ranking of solution toxicity of different metalic ions was established on the basis of the concentration producing 50% yield loss: Mn<B<Fe<Zn<Al<Ga<La<Sc<Cu (Wheeler et al., 1995). Cultivated plants differ significantly in their response to Al toxicity: pea<two-rowed barley<oat<rye<rice (Slaski, 1994). Therefore aluminium ions have the potential to limit crop growth and yield.

Absorbed into cells, Al produces different morphological changes: 1) disruption, disfunctionality and blockage of Ca^{2+} channels (Rengel, 1995); 2) inhibition of DNA replication (Rengel, 1992); 3) deficiency in oxidative capacity of mitochondria (De Lima et al., 1994); 4) increased vacuolation, loss of turgor of meristematic cells (De Lima et al., 1994); 5) reduced chlorophyll, transpiration, photosynthesis (Ohki et al., 1984).

Based on the distinction between external (exclusion) and internal resistance mechanisms - either on the site of metal detoxification or immobilisation in the apoplasm/symplasm - different mechanisms involved in Al tolerance were proposed.

External resistance mechanism (exclusion of Al ions) consists in limiting the rate of Al transport across the plasma membrane and cytosol. Extracellular, including extracytosolic lesions cause disruption of normal functioning of the plasma membrane. Although, several external mechanisms, taking into account the whole plant characteristics were described in the literature, resistance to Al ions seems to be mediated ultimately by internal processes of the cells.

All curent hypotheses regarding internal resistance to Al are generally based on several mechanisms: 1) chelation in the cytosol; 2) compartmentation in the vacuole; 3) evolution of Al tolerant enzymes or 4) increased enzyme activity.

MATERIALS AND METHODS

Plant material was represented by 24 genotypes (Table 1), released in Romania (six), Germany (six) and USA (twelve).

1.1. Relative root elongation rates (RRE%) of plants exposed to Al ions. A number of 72 seeds per genotype was surface sterilized for 20 minutes in a 5% (vol/vol) NaClO solution (containing 1-2 ml Tween 20), rinsed three times with distilled water and germinated asepticaly for 64 hours on Anchor paper moistened with 0.2 mM $CaCl_2$ solution at dark and 22°±1°C.

Individual seedlings with uniform size were transfered in 15 mm/60 mm opaque tubes. Each bottom of the tubes was covered with a nylon screen (with a square opening of 0.3 cm) secured with silicone glue. The planting tray contained 8 x 9 opaque tubes, hanged up on a support and placed in a container with 9 l aerated nutrient solution. When the screen was pushed into contact with the solution, surface tension forces maintaned the contact. The nutrient solution had the following composi-

Table 1. The biologic material tested during the preliminary stage of Al - tolerance screening

WINTER SIX-ROWED BARLEY	WINTER TWO-ROWED BARLEY
•Dana	• Fundulea 1019 / 86
• Fundulea 468 / 86	• Fundulea 1385 / 90
• Fundulea 663 / 85 (PRECOCE)	• Andra
• Dayton	• Poland
• Missouri early beardless	• Igri
• Olimpia	• Corona
• Smooth awn	• Franka
• Sunrise	
• Tennessee winter	
• Winter club	
• Wisconsin winter	
SPRING SIX-ROWED BARLEY	SPRING TWO-ROWED BARLEY
• Golden promise	• Gull
	• Volla
	• Dissa
	• Kenia
	• Bavaria

tion: 0.4 mM NH_4NO_3; 0.1 mM $(NH_4)_2SO_4$; 2.5 mM $MgCl_2$; 6.5 mM KNO_3; 4 mM $CaCl_2$.

Aproximately 20 ml nutrient solution per seedlings was maintained permanentely in the container during the experiments.

For Al treatments, Al stock solution was added dropwise after pH had been lowered to 4.1 (for 74 µM total Al) or 4.2 (for 148 µM total Al treatment). During plant growth, pH of nutrient solutions was adjusted daily by 0.2N KOH or 0.2N HCl. The pH generally remained within the 3.9 to 4.1 range over the entire growing period. The Al stock solution reprensented by 0.1M $AlCl_3$ x $6H_2O$ solution.

After 5 day Al treatment, the seminal root of the seedlings (and also control seedlings) were measured. In order to visualize differences between cultivars in tolerance to acid pH and Al ions, the initial length (Li) and final length (Lf) of seminal roots were measured. Using the relative root elongation (RRE%) rate relevated three Al tolerance level in the response of analysed genotypes.

$$RRE\% = \frac{\text{L. final exp.} - \text{L. initial exp}}{\text{L. final control} - \text{L. final control}}$$

RRE: 0 - 40%: the lowest Al tolerance level;

RRE: 41 - 70%: the medium Al tolerance level;

RRE: 71 - 100%: the highiest Al tolerance level.

Concomitantly, using a fotonic microscope and seedlings disposed on filter paper (moistened with nutritive solution without/with Al^{3+}) were observed the elongation of each central seminal root.

1.2. Hematoxylin forms chemical complexes in Al - treated barley roots meristem. Seed germination and seedling growth conditions were described in 1.1. Where seed dormancy was observed imbibed seeds were placed in the refrigerator at 4°C for three days.

The staining solution consisted of 2 g/l hematoxylin and 0.2 g/l $NaIO_3$ disolved in a liter of distilled water as recommended by Polle et al., 1985.

The seedlings were grown in nutrient solution for two days and then they were transferred in three containers containing aerated nutrient solution with: 1) 0.03 mM Al^{3+}; 2) 0.06 mM Al^{3+}; 3) 0.09 mM Al^{3+}. After 17 hours (light, at 22°C), the nutrient solution were replaced with aerated distilled water for 30-60 minutes, respectively with 0.2% hematoxylin solution for 15 minutes. Enough hematoxylin solution was added (with ocasionally agitation) to cover the roots. After staining, the plantles were washed out (Havas, 1986) and maintained for 30 - 45 minutes in aerated distilled water (Polle et al., 1985). The experiment was three times repeted and a bifactorial analysis of variance was performe where the factor A was the genotype and factor B represented Al concentration (Săulescu, and

Săulescu, 1967). Relative hematoxylin stainability - G - was defined with the ecuation:

$$G\% = \frac{(C_1 + C_2 \times 0.5) \times 100}{N}$$

were: N - total number of analysed roots per aluminium concentration level;

C_1, C_2 - root growth meristem number stained 100% respectively, 50% hematoxylin.

1.3. A redox potential exists at the root surface. 24 seeds per genotype proceeding each one from cultivars and lines with different Al tolerance level (sensitive / medium / tolerant) were sterilised and germinated on filter paper moistened with 0.25 mM $CaCl_2$ solution. The three-days-old plantlets were transferred in treatment solution containing 10 mg Ca^{2+}/L, 0.6 mg Al^{3+}/L, (pH = 4.6) respectively, in control solution containing only 10 mg Ca^{2+}/L (pH = 4.6). In the proper experiment 24 three-days-old seedlings per genotype with root lengths of $\approx$10 cm were grown for 24 hours in opaque test tubes containing 20 mL of deionized water, augmented with 10 mg Ca^{2+}/L, 0.43 mM $K_3Fe(CN)_6$/L (pH = 4.6). Ferricyanide concentrations were determined by absorbance at 420 mm. The control tubes were considered the tubes without roots (seedlings). Change in H^+ concentration were determined directly from pH measuring. It was calculated correlation coeficient, standard deviation of differences and standard deviation of regression coeficient.

1.4. The number of cells in mitosis after exposure to Al of root meristem. 24 seed per genotype, proceeding each one from cultivar or lines with different Al tolerance level (sensitive/medium/tolerant) were sterilised and germinated for two days on filter paper moistened with distilled water. After this period they were placed onto filter paper soaked in Hoagland solution supplemented by 1.25 mM hydroxyurea - HU. The seedlings were rinsed out three times with distilled water (Nkongolo and Klimaszewska, 1995) and removed again on filter paper moistened with Hoagland solution without HU. After five hours, the seedlings were again placed on a filter paper soaked with Hoagland solution supplemented by 1.25 mM colchicine (0.05%, vol/vol) for 4 hours.

After rinsing five times with distilled water, the roots were kept in ice water for 24 hours, rinsed three times with 95% ethanol (for 5 minutes) and fixed with 70% ethanol. The roots were then removed at -20°C (for two hours). With the purpose of rehidration, the roots were three time rinsed with distilled water (for 5 minutes). After the fixative was rinsed away with distilled water, the primary roots tips were collected in Petri dishes filled with distilled water. The digestion of meristems was carried out using an enzime mixture (2.5% pectolyase Y 23; 2.5 cellulase "Onozuka R-10"). The digestion time was 30-40 minutes after that the root were removed in 70% ethanol (for one hour). The determination of mitotic index was carried out using the squash method, with 65% acetic acid (Pan et al., 1992).

In order to Al treatment, 15 seedlings / genotype / variant were exposed to 74 µM Al^{3+} for 0; 2; 4; 6; and 24 hours using the method 1.1. Then, the roots were rinsed out with distilled water and the treated seedlings were handled like the control.

The ponderate index of classification (PIC) served to dates analyse.

$$PIC = \frac{100}{V_0} \sum_{i=t.\min}^{t.\max} \left(Vi \frac{i}{t.\max} \right)$$

were: V - mitotic index after the time "i"

$i \in (2, 4, 6, 24)$

$$PIC = \frac{V_{2h}}{V_0} \bullet \frac{2}{6} \bullet 100 + \frac{V_{4h}}{V_0} \bullet \left(\frac{4}{6}\right) \bullet 100 + \frac{V_{6h}}{V_0} \bullet \left(\frac{6}{6}\right) + \frac{V_{24h}}{V_0} \bullet \left(\frac{24}{6}\right)$$

RESULTS AND DISCUSSIONS

1.1. The effect of Al ions and acid pH on the morphogenesis - reckoning the root elongation rate (RRE) of Al treated plants.

The obtained data for 24 genotypes (Figure 1) showed difference in the response to low pH and aluminium ions.

In RRE calculation were used only data from the 74 µM Al^{3+} experiment. According to

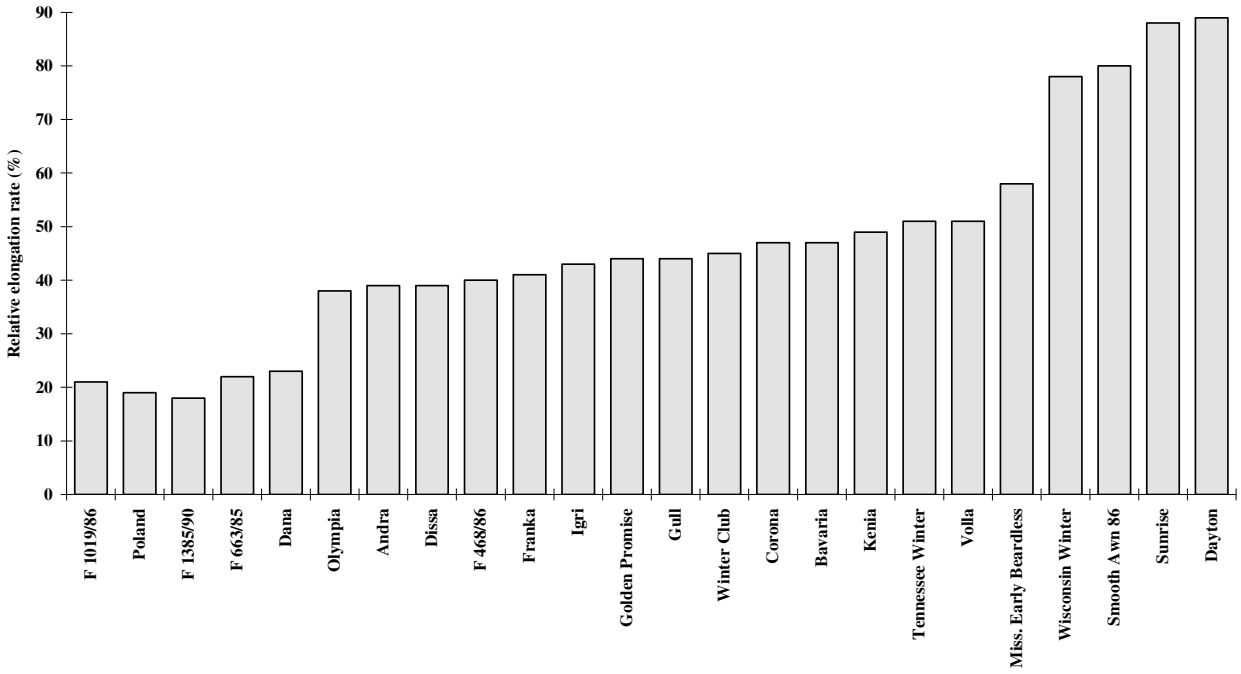

Figure 1. Relative elongation rate for 24 genotypes of six- and two rowed barley

our results, nine barley genotypes were included in the lowest Al tolerance class (RRE: 0 - 40%), twelve genotypes in the medium Al tolerance level (RRE: 41 - 70%) and three genotypes ranked in the highiest Al tolerance level (RRE >71%). Of the screened cultivars only Dayton, Smooth Awn 86 and Sunrise recorded very close RRE value. Aluminium values from the assay were used to calculate ionic activities and Al speciation by a modified GEOCHEM computer program (Rengel, 1989): 12.5 and 25 μM Al^{3+} for 74 and 148 μM of total Al in nutrient solution. The relative length of Al - treated barley roots of sensitive cultivar was 1/3 to 1/2 of the control. Excepted for Dayton, Smooth Awn 86 and Sunrise, the Al - treated seedling recorded a steadily decreased growth when it was removed to pots with normal soil. The occurrence of different phenotypes was observed:

- mostly semidwarf plant types;
- shortened internodes;
- the leaves of some seedlings expressed white or yellow longitudinal stripes.

Rengel, 1995, considered that sometime low Al concentration may stimulate plant growth owing to improvement of Fe and P nutrition, alteration in the distribution of growth regulators, alleviation of P - toxicity or prevention of Cu and Mn toxicities.

Using a fotonic microscope it was monitorized the elongation of the central seminale roots for Dayton cultivar. The growth rate of roots was not affected only for the first three hours of Al - treatment.

1.2. The Al response of barley genotypes assayed by the hematoxylin staining procedure. The hematoxylin scores of analysed genotypes showed in table 2 and figure 2. The hematoxyline scores ranged from complete staining at all concentration, to no staining at 0.03 mM Al or partial staining at 0.06 and 0.09 mM Al.

The Al sensitive cultivars proved high values of G% while the Al tolerant ones displayed reduced values of G%. Three genotypes revealed at first Al concentration (0.03 mM Al^{3+}) a high hematoxylin stainability (G>55%), indicating a reduced Al tolerance. The roots of all cultivars developed a readily stainable zone in the apical region.

Table 2. Al - hematoxyline complexes formation on the root meristem surface

Genotype	Hematoxylin stainability of Al - treated roots (Angular transformation)			Mean per genotype	Duncan test[1]
	0.03 mM Al	0.06 mM Al	0.09 mM Al		
Dayton	0	21.92	72.61	31.51	A
Smooth Awn	0	23.99	69.27	31.01	A
Sunrise	0	27.09	71.10	32.73	A
Volla	0	39.82	66.55	35.48	B
Gull	0	40.15	65.95	35.36	B
Bavaria	0	43.37	62.38	35.25	B
F 468 - 86	58.12	66.47	81.61	68.73	C
F 1385 - 90	62.96	77.77	82.29	76.34	D
Andra	60.17	81.49	88.29	76.65	D
Mean	20.13a[1)]	46.87 b	73.97 c	-	

1) Means without common letters are significantly different at $P \leq 0.05$

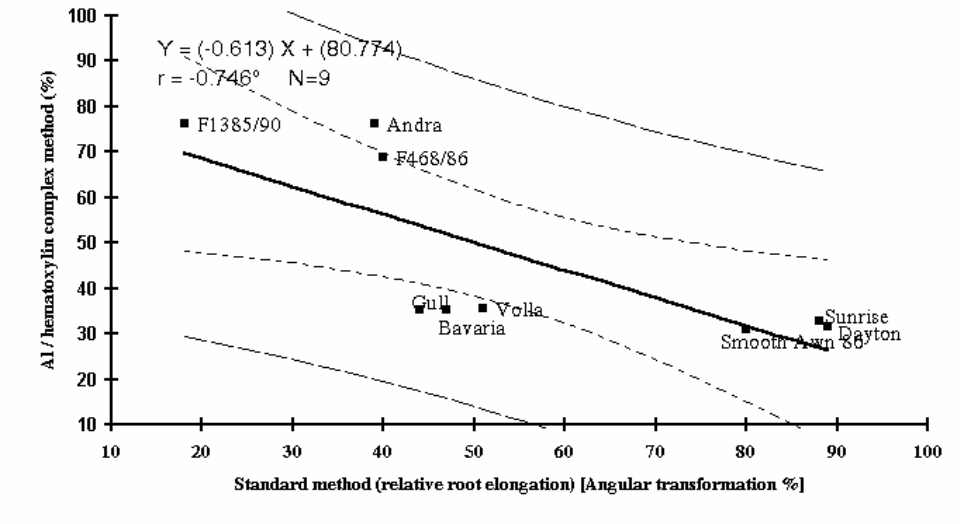

Figure 2. Relationship between standard and the Al / hematoxylin complex methods

The second Al concentration (0.06 mM Al) is correlated with Al - hematoxyline complexes (hemateine) formation at all tested genotypes with different G values. The genotypes classified previously as Al - sensitive recorded now G% values highier three until four time than Dayton. At low Al concentration the stainable region was unstained close to the root tip. As the concentration of Al increased, more of the root was stained and the unstained zone became smaller until a continous apical stained region (of 0.6 - 1 cm) was formed. Because the second Al concentration (0.06 mM Al^{3+}) allows a very efficient screening of Al - hematoxyline complexe formation, it would be necessary to use only lower Al concentration (0.04 mM Al; 0.05 mM Al). At third Al concentration (0.09 mM Al) all genotypes recorded very significantly differences in G% value (G>62%).

Numerous autors examined factors that might cause Al to be selectivity immobilized on the root surface of Al - sensitive cultivars and react there with hematoxylin:

- the presence of extracellular phosphate (*in vitro* Al and phosphate formed a hematoxyline - binding precipitate when the P: Al ratio was greater than 1.0 (Hammond, 1994; Nichol, 1993);

- acid pH of medium (Putteril, 1988; Havas, 1986);

- the abundant uronic acids from cell walls and the root cation exchange (De Lima, 1994; Ownby, 1993).

1.3. Redox potential determination on the roots surface

Genotypes differing in seedling Al tolerance were identified (Table 3 and Figure 3). The determination of the redox potential (with reduced nicotinamide adenine dinucleotide as the proposed electron donor and the nonpenetrating ferricyanide ions serving as the electron acceptor) releved that the selection for increased Al tolerance was associated with a lower rate of ferricyanide reduction. Al - sensitive genotypes tend to create lower pH (high H^+ release) than Al tolerant genotypes. The analysed genotypes were therefore classified in three groups:

- Al - tolerant, which were associated with reduced rates of ferricyanide and H^+ release (Dayton, Smooth Awn 86, Sunrise);
- Al - sensitive, which were associated with high rate of ferricyanide and H^+ release (F 1385 -90, F 468-86, Andra);
- Al - moderately sensitive, which were associated with mean value of the analysed parameters (Volla, Bavaria, Gull).

Table 3. Root characteristics associated with Al - tolerance for 9 two- and six-rowed barley genotypes

Genotype	Ferricyanide reduction rate (mmol/m^2.s) - x -	H^+ release (mmol/m^2.s) - y -	Regression reckoning Ax + B = Y
F 1385 - 90	6.62 ± 0.55	2.62 ± 0.13	Standard deviation for x variable = 1.47
F 468 - 86	3.83 ± 0.51	2.53 ± 0.09	Standard deviation for y variable = 0.77
Andra	4.17 ± 0.47	2.12 ± 0.11	Correlation coefficient = 0.91**
Dayton	2.45 ± 0.36	0.81 ± 0.07	DL 5% = 2.74
Sunrise	2.83 ± 0.35	0.63 ± 0.05	1% = 4.20
Smooth Awn	2.91 ± 0.36	0.72 ± 0.06	Probe T (Student) = 5.94
Volla	4.90 ± 0.38	1.85 ± 0.09	
Gull	4.83 ± 0.31	1.23 ± 0.05	
Bavaria	4.23 ± 0.33	1.04 ± 0.03	

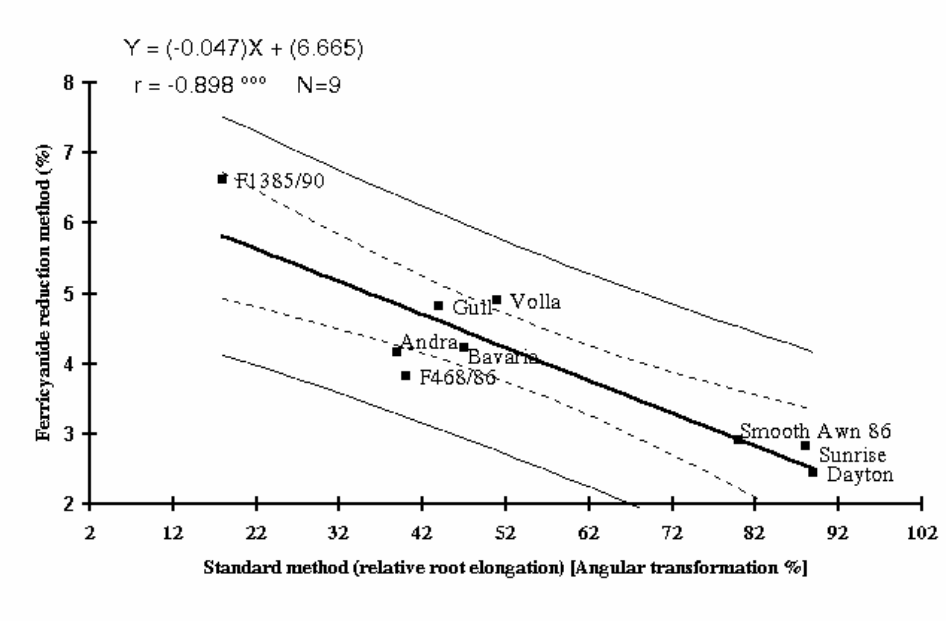

Figure 3. Relationship between the standard and the ferricyanide reduction methods

1.4. Mean mitotic index determination in Al - treated roots

The mitotic cycle duration in two-rowed barley (*Hordeum distichum*, 2n = 14) and six-rowed barley (*Hordeum vulgare*, 2n = 14) is 12 hours (Anghel and Raicu, 1983).

Root meristem represent the most used material for plant cytogenetics. Because of a low natural frevency of synchronously dividing cells (Anghel and Raicu, 1983) and in order to enhance significantly the mitotic index

Table 4. Mitotic index (Angular transformation values) of Al - treated barley roots

Genotype	Al treatments of roots meristems (hours)				Mean per genotype	Duncan test [1]
	0h	2h	4h	6h		
Dayton	30.24	28.15*	17.50***	10.72***	21.66	A[1)]
Smooth Awn	31.37	30.46	18.99***	9.33***	22.54	A
Sunrise	29.53	27.25**	16.84***	9.07***	20.68	A
Bavaria	27.54	18.45***	11.53***	8.04***	16.39	B
Gull	28.79	20.61***	11.53***	0	15.24	B
Volla	29.99	19.39***	10.93***	0	15.08	BC
F 468 - 86	29.91	18.31***	9.63***	0	14.59	BC
F 1385 - 90	28.79	17.85***	9.45***	0	14.03	BC
Andra	30.25	16.42***	0	0	11.67	C
Mean	29.60 a[1)]	21.94 b	11.83 c	4.13 d	-	

1) Means without common letters are significantly different at $P \leq 0.05$
*, **, *** - significantly different to control (untreated roots), for $P<0.05$, $P<0.01$ respectively $P<0.001$ in accordance with Student test.

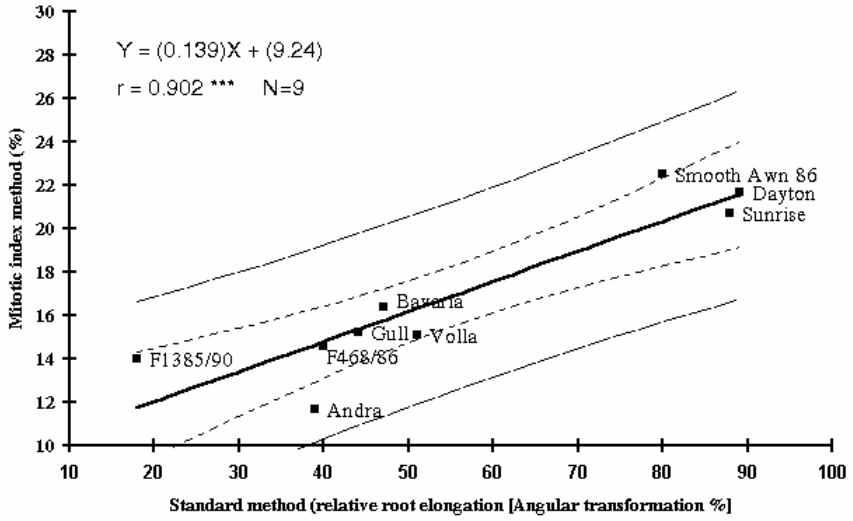

Figure 4. Relationship between the standard and the mitotic index methods

IM%, we utilised a pretreatment with 1.25 mM hydroxyuree (HU).

Table 4 and figure 4 show mitotic index values in Al - treated roots.

A two-hours Al - treatment of barley roots produced a significantly decrease of mitotic index.

A four or six-hours Al - treatment of barley roots reduced to 1/2 respectively 1/7 the IM values. Smooth Awn cultivars was the only one who didn't reduced significantly IM% after two hours Al - treatment (indicating a high Al - tolerance level).

It can be seen from table 4 that the analysed genotypes classified in three groups (using the Duncan test):

- relative tolerant genotypes;
- medium sensitive genotypes;
- sensitive genotypes.

The two hours Al - treatment can offers enough data (comparatively with 4; 6; 24 Al - treatment).

CONCLUSIONS

Genetic variability for Al tolerance as measured by four seedling screen was found in the 24 analysed genotypes. Additional screening of these genotypes is needed.

Dayton, Smooth Awn and Sunrise cultivars classified as Al - tolerant genotypes.

The primary target of Al ions is the meristematic zone of roots with the wall cells as principal situs of Al action. Differences were

found in tolerance of barley cultivars depending on their origin.

Irreversible inhibition of root growth at a particular concentration of Al was associated with an increase in Al concentration of the roots.

Sensitive genotypes registered maximum valus for hematoxylin stainability at 0.09 mM Al^{3+}.

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WINTER SIX-ROWED BARLEY (OT)
- DANA
- FUNDULEA 468 / 86
- FUNDULEA 663 / 85 (PRECOCE)
- DAYTON
- MISSOURI EARLY BEARDLESS
- OLIMPIA
- SMOOTH AWN
- SUNRISE
- TENNESSEE WINTER
- WINTER CLUB
- WISCONSIN WINTER

WINTER SIX-ROWED BARLEY (OP)
- GOLDEN PROMISE

WINTER TWO-ROWED BARLEY
- FUNDULEA 1019 / 86
- FUNDULEA 1385 / 90
- ANDRA
- POLAND
- IGRI
- CORONA
- FRANKA

SPRING TWO-ROVED BARLEY
- GULL
- VOLLA
- DISSA
- KENIA
- BAVARIA

Table 2. Al - hematoxyline complexes formation on the root meristem surface.

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Bavaria	0	43.37	62.38	35.25	B
F 468 - 86	58.12	66.47	81.61	68.73	C
F 1385 - 90	62.96	77.77	82.29	76.34	D
Andra	60.17	81.49	88.29	76.65	D
Mean	20.13a[1)]	46.87 b	73.97 c	-	

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Andra	30.25	16.42***	0	0	11.67	C
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***, **, *** - significantly different to control (untreated roots), for P<0.05, P<0.01 respectively P<0.001 in accordance with Student test.**

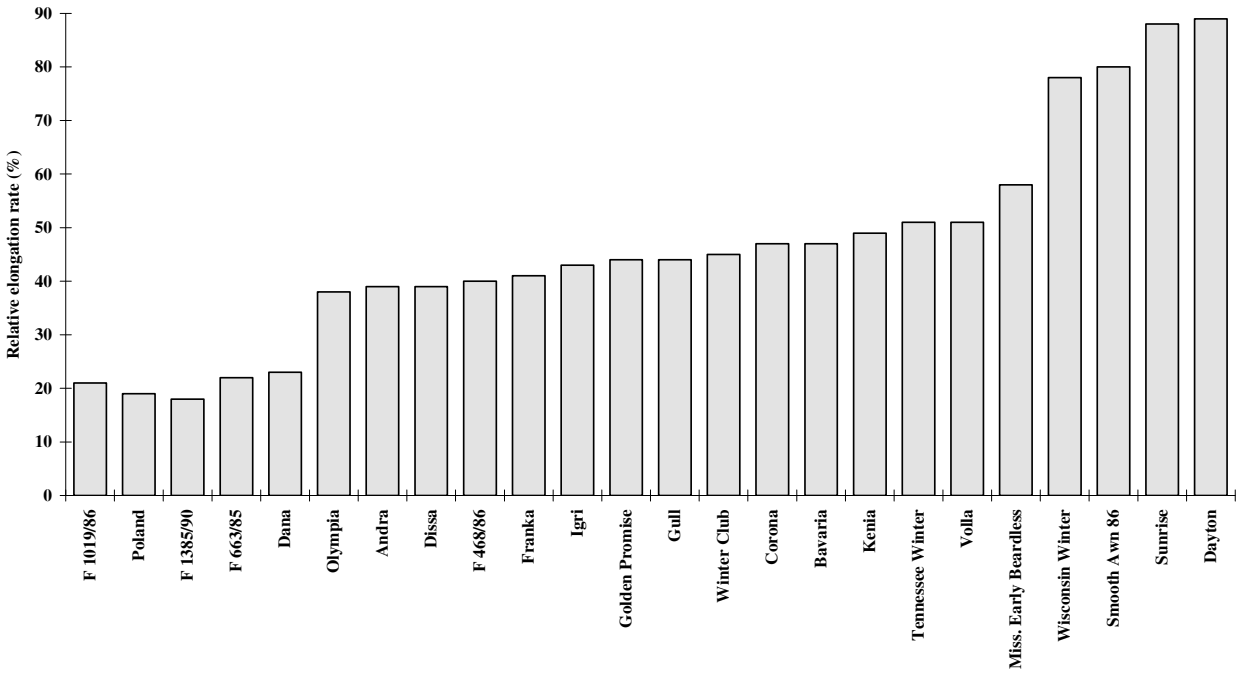


Figure 1. Relative elongation rate for 24 genotypes of six- and two rowed barley

Figure 2. Relationship between standard and the Al / hematoxylin complex methods

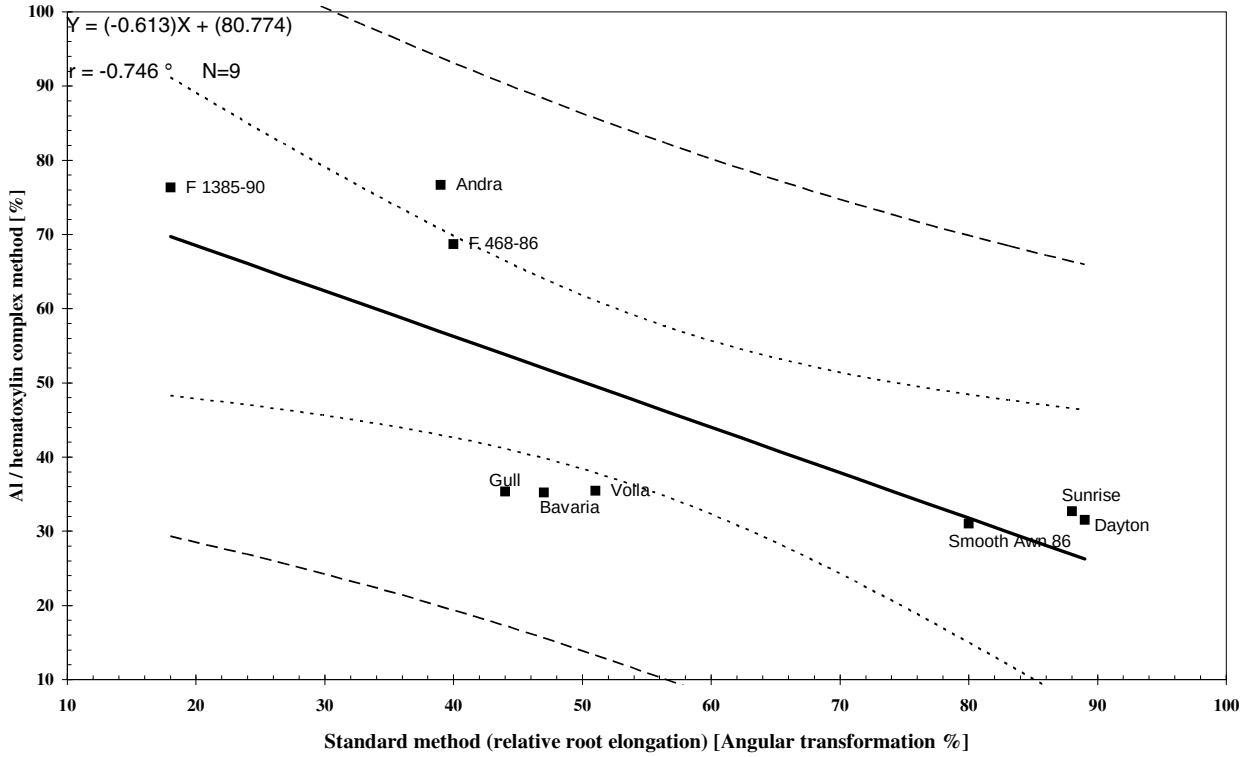


Figure 3. Relationship between the standard and the ferricyanide reduction methods

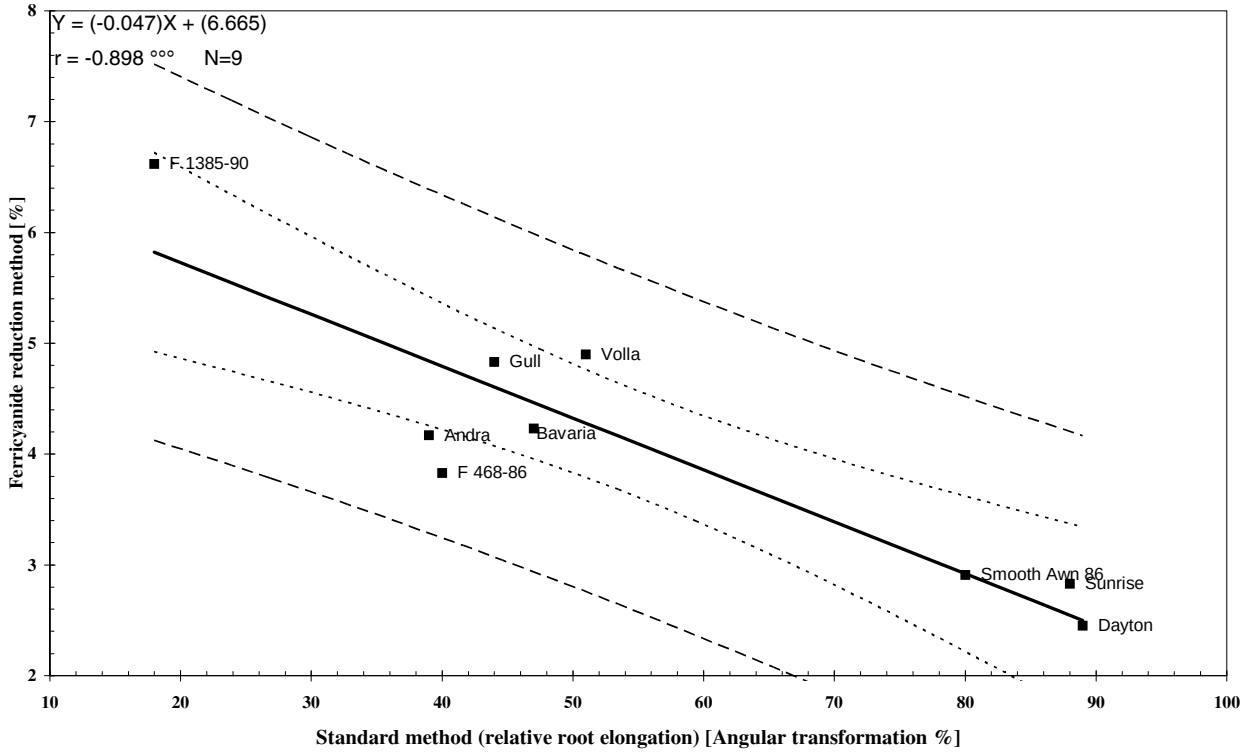
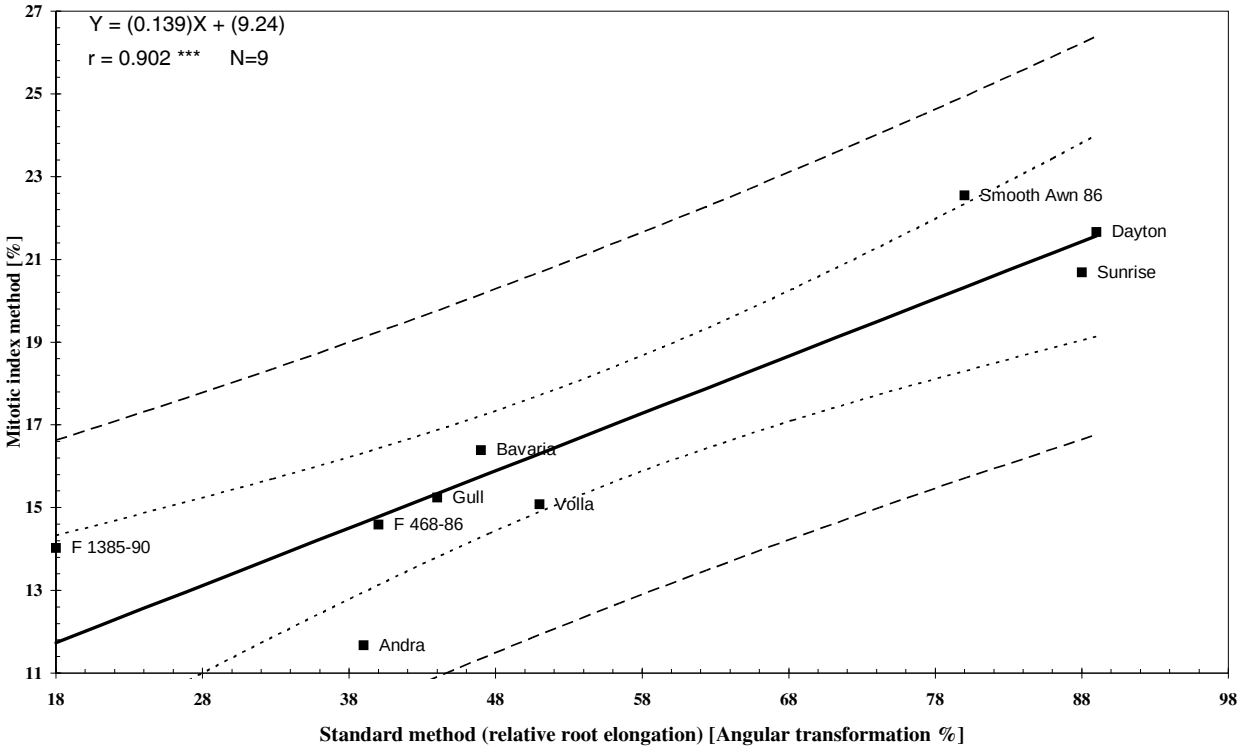


Figure 4. Relationship between the standard and the mitotic index methods



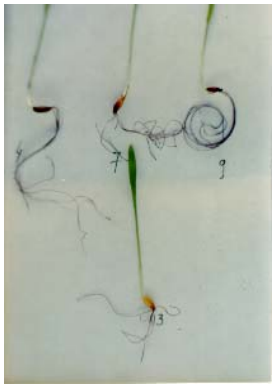


Table 2. Al - hematoxyline complexes formation on the root meristem surface.

Genotype	Hematoxylin stainability of Al - treated roots (Angular transformation)			Mean per genotype	Duncan test[1]
	0.03 mM Al	0.06 mM Al	0.09 mM Al		
Dayton	**0**	**21.92**	**72.61**	**31.51**	**A**
Smooth Awn	**0**	**23.99**	**69.27**	**31.01**	**A**
Sunrise	**0**	**27.09**	**71.10**	**32.73**	**A**
Volla	**0**	**39.82**	**66.55**	**35.48**	**B**
Gull	**0**	**40.15**	**65.95**	**35.36**	**B**
Bavaria	**0**	**43.37**	**62.38**	**35.25**	**B**
F 468 - 86	**58.12**	**66.47**	**81.61**	**68.73**	**C**
F 1385 - 90	**62.96**	**77.77**	**82.29**	**76.34**	**D**
Andra	**60.17**	**81.49**	**88.29**	**76.65**	**D**
Mean	**20.13a[1)]**	**46.87 b**	**73.97 c**	**-**	

1) Means without common letters are significantly different at P≤ 0.05

Figure 2. Relationship between standard and the Al / hematoxylin complex methods

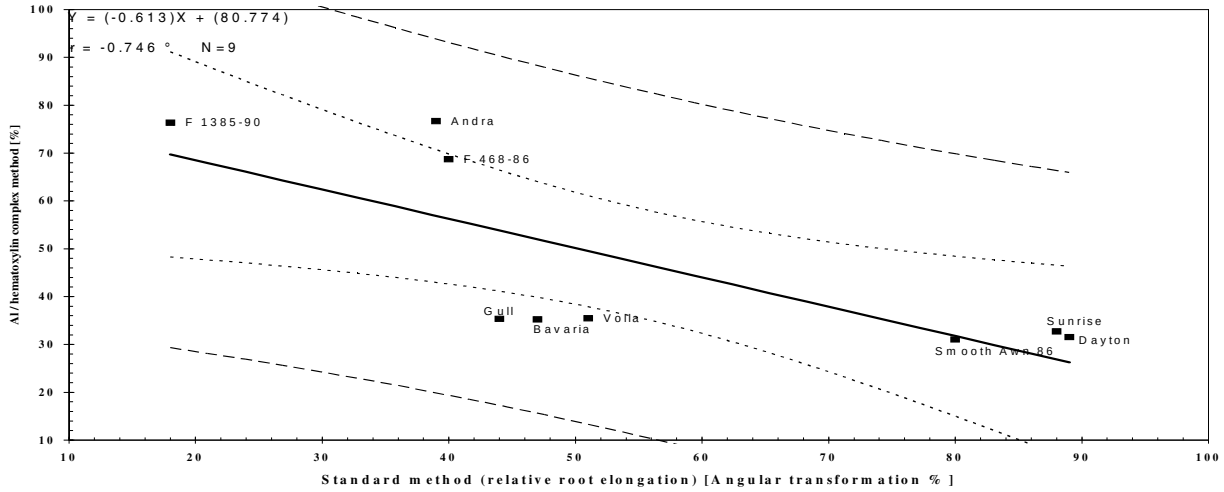


Table 3. Root characteristics associated with Al - tolerance for 9 two- and six-rowed barley genotypes

Genotype	Ferricyanide reduction rate ($mmol/m^2.s$) - x -	H^+ release ($mmol/m^2.s$) - y -	Regression reckoning Ax + B = Y
F 1385 - 90	6.62 ± 0.55	2.62 ± 0.13	Standard deviation for x variable = 1.47
F 468 - 86	3.83 ± 0.51	2.53 ± 0.09	Standard deviation for y variable = 0.77
Andra	4.17 ± 0.47	2.12 ± 0.11	Correlation coefficient = 0.91**
Dayton	2.45 ± 0.36	0.81 ± 0.07	DL 5% = 2.74
Sunrise	2.83 ± 0.35	0.63 ± 0.05	1% = 4.20
Smooth Awn	2.91 ± 0.36	0.72 ± 0.06	Probe T (Student) = 5.94
Volla	4.90 ± 0.38	1.85 ± 0.09	
Gull	4.83 ± 0.31	1.23 ± 0.05	
Bavaria	4.23 ± 0.33	1.04 ± 0.03	

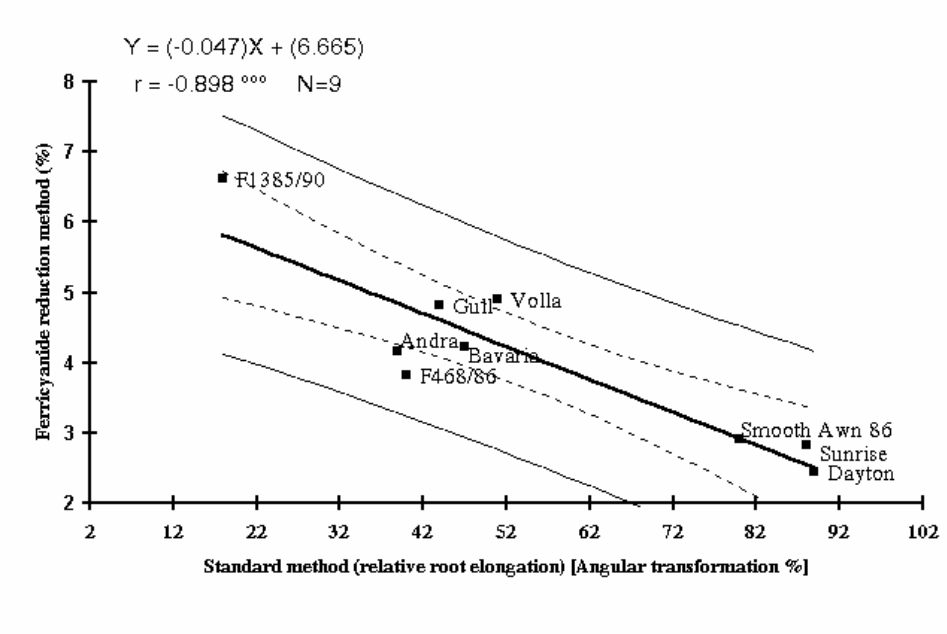


Figure 3. Relationship between the standard and the ferricyanide reduction methods

Table 4. Mitotic index (Angular transformation values) of Al - treated barley roots

Genotype	Al treatments of roots meristems (hours)				Mean per genotype	Duncan test [1]
	0h	2h	4h	6h		
Dayton	30.24	28.15*	17.50***	10.72***	21.66	A[1)]
Smooth Awn	31.37	30.46	18.99***	9.33***	22.54	A
Sunrise	29.53	27.25**	16.84***	9.07***	20.68	A
Bavaria	27.54	18.45***	11.53***	8.04***	16.39	B
Gull	28.79	20.61***	11.53***	0	15.24	B
Volla	29.99	19.39***	10.93***	0	15.08	BC
F 468 - 86	29.91	18.31***	9.63***	0	14.59	BC
F 1385 - 90	28.79	17.85***	9.45***	0	14.03	BC
Andra	30.25	16.42***	0	0	11.67	C
Mean	29.60 a[1)]	21.94 b	11.83 c	4.13 d	-	

1) Means without common letters are significantly different at $P \leq 0.05$
*, **, *** - significantly different to control (untreated roots), for P<0.05, P<0.01 respectively P<0.001 in accordance with Student test.

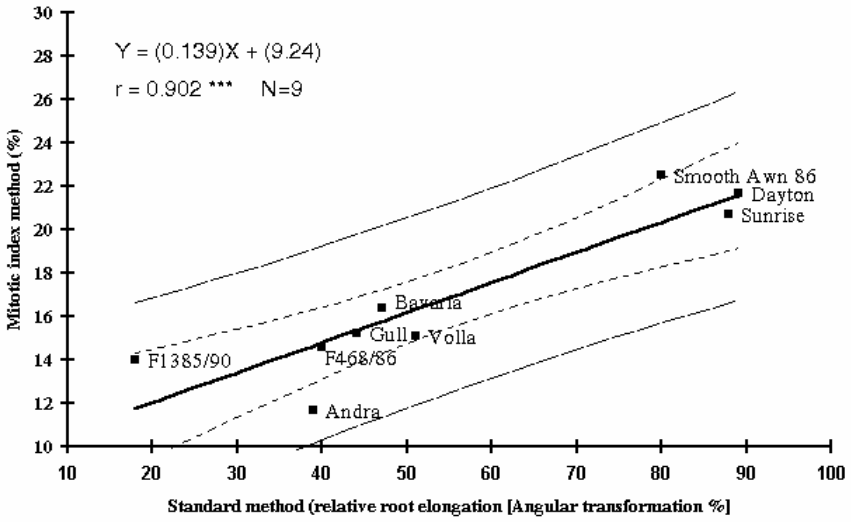


Figure 4. Relationship between the standard and the mitotic index methods