Plants Tolerance To Heavy Metals (Cd, Ni, Pb) – Case Study *Salix* Sp.

Corneanu Mihaela1, Hernea Cornelia1, Butnariu Monica1, Corneanu Gabriel2, Sărac Ioan1, Hollerbach Wilhelm3, Neţoiu Constantin4, Petcov Andreea Adriana1

[1]USAMVB Timisoara, Faculty of Horticulture and Forestry, Genetic Engineering Dept., Timisoara, Romania, e-mail: micorneanu@yahoo.com;

[2]University of Craiova, Faculty of Agriculture and Horticulture, Craiova, Romania;

[3]REBINA Agrar SRL; Timişoara, Romania

[4] Forest Research and Management Institute Bucharest,

Introduction

- The species of *Salix* genus, constitute a promising source in the action of fighting against the environment degradation, and offer remedy for about two third from the all degradation types.
- The majority of the willow species, present a good adaptation to hypoxic conditions, feature which suggest that they manifest a preference for mineral nutrition in comparison with organic one.
- Thus, many of willow species can be developed on soils with a big amount of minerals and/or radionuclides, being both phytoremediatory species, as well as pioneer ones, contributing to the soil restoration.

- Thus, the willow species, posses the capacity for development in degraded areas, natural or anthropic, as swamps, abandoning crops areas, sandy dune, riparian sandy areas, gravels, a.o.
- In this paper are present some laboratory comparative tests of heavy metals tolerance on four *Salix* sp. genotypes

Material and Methods

- **Biological material**:

  - clone 202 (*Salix alba*), hybrid 892 (*Salix alba*);
  - Inger (*Salix viminalis*) and Gudrun (*Salix viminalis*).

- The genotypes of *Salix alba* are native from Romania, produced in the Forest Research and Management Institute Bucharest, while the genotypes of *Salix viminalis* are native from Sweden, but the plant material was produced under license in Romania by REBINA Agrar SRL.

- As plant material were used one-year-old cuttings (5-10 cm long), with 2-6 buds each.

Experimental design

- There were ten experimental variants for each genotype: three concentrations of Cd, Ni, Pb and Control (tap water).

Metal	A (ppm/l)	B (ppm/l)	C (ppm/l)
Cd	1.0	3.0	6.0
Ni	50.0	150.0	450.0
Pb	50.0	150.0	450.0

Per genotype, per heavy metal and each of three concentration, five replication were used (5 cuttings/replication).

The cuttings were maintained in solutions for 17 days. In the days 7th and 17th, were performed biometrical observations on: the roots number and length, the shoots number and length, the leaves number/shoot, viability of the shoots.

Methods

- *Biometrical observations*

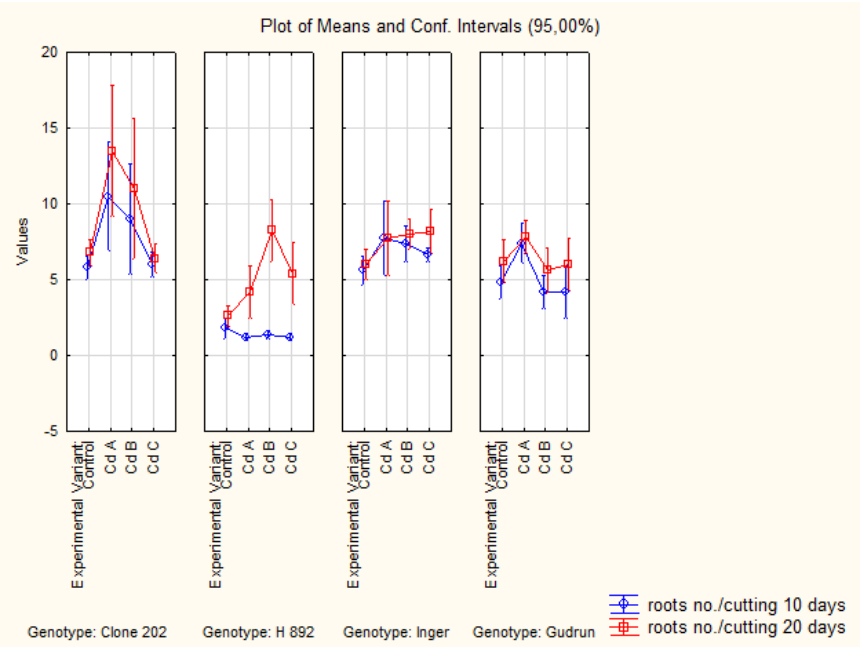
- The cuttings were maintained in solutions for 17 days. In the days 7th and 17th, were performed biometrical observations on: the roots number and length, the shoots number and length, the leaves number/shoot, viability of the shoots.

- *Cytological investigation*
- Roots for cytological investigations (0.8-1cm length) were harvested after 24 hours of treatment with heavy metals solutions (a complete mitotic cycle) and analyzed by optical microscopy.
- The cytogenetic observations, on fresh slides, were performed to Olympus BO71 BH2RFCA optical microscope and the microphotographs were taken using Cell F imaging software.
- Mitotic index (MI) was calculated as the ratio between the number of cells in mitosis (prophase + metaphase + anaphase + telophase) and the total number of cells.

- *Statistics*
- All statistical analyses were performed with commercially available software (STATISTICA 10).
- The data were analyzed one-way analysis of variance (ANOVA) , Duncan test and correlation coefficient. The differences were considered significant at a probability level of 95% ($P < 0.05$).

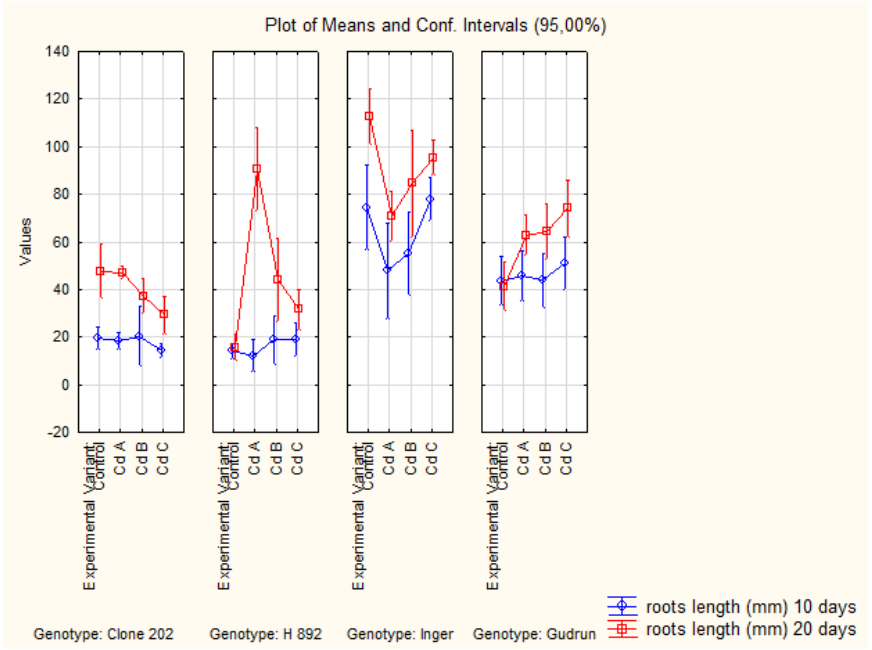
RESULTS AND DISSCUSIONS

Rhysogenesis process
Cd



Analysis of Variance (exp Salix Cd) Marked effects are significant at p < ,05000

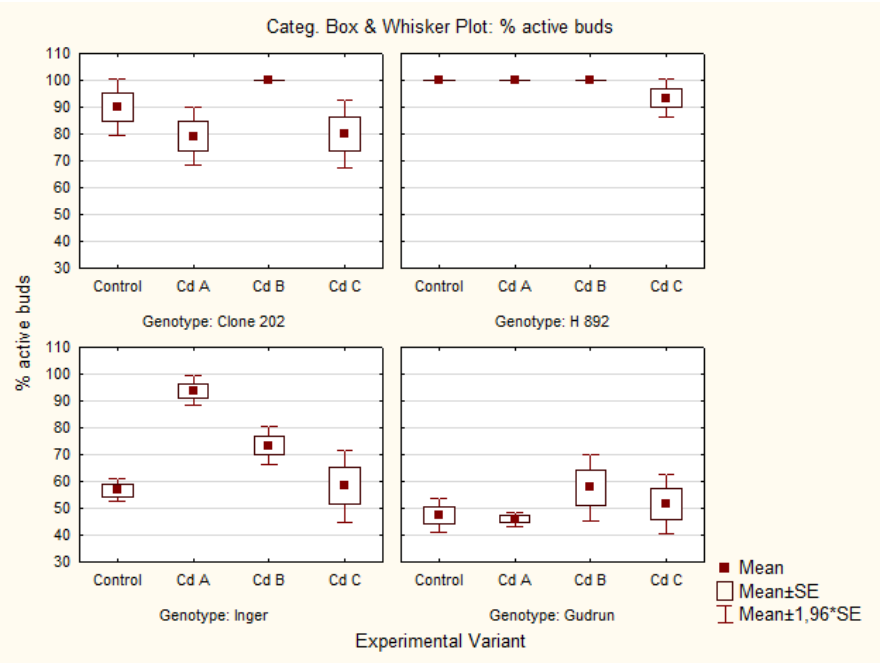
	SS - Effect	df - Effect	MS - Effect	SS - Error	df - Error	MS - Error	F	p
roots no./cutting 10 days	215,256	3	71,752	3927,5	241	16,297	4,402810	0,004890
roots no./cutting 20 days	380,746	3	126,915	4836,0	241	20,067	6,324705	0,000381
roots length (mm) 10 days	3325,882	3	1108,627	200569,0	241	832,237	1,332106	0,264532
roots length (mm) 20 days	6311,518	3	2103,839	266742,1	241	1106,814	1,900807	0,130069



H 892

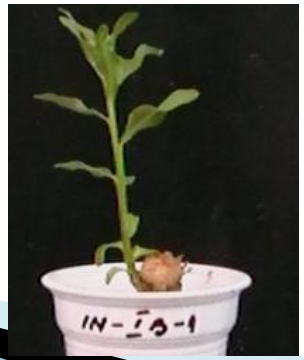
Cd stimulate roots meristems differentiation, and slightly the cell division and elongation

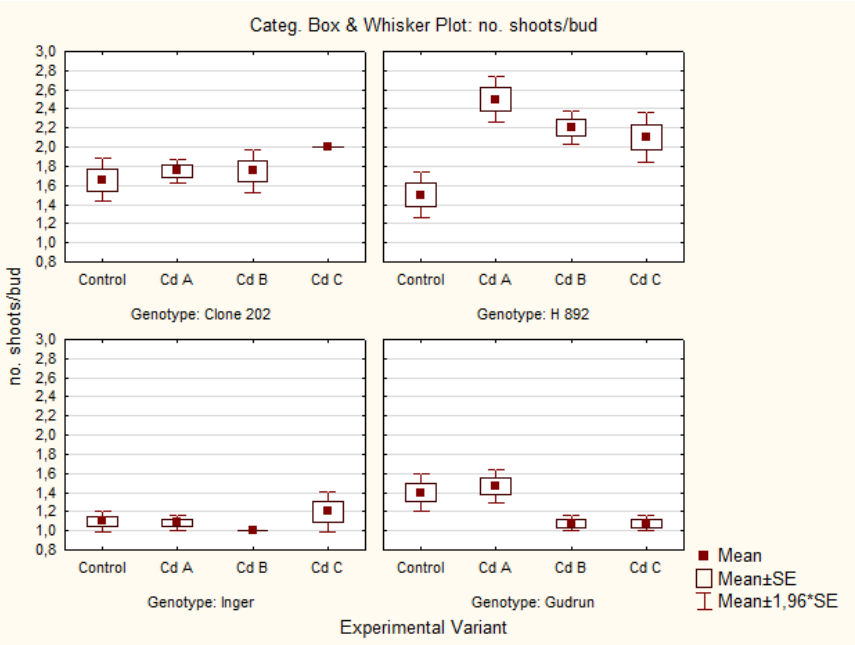
Shooting process Cd



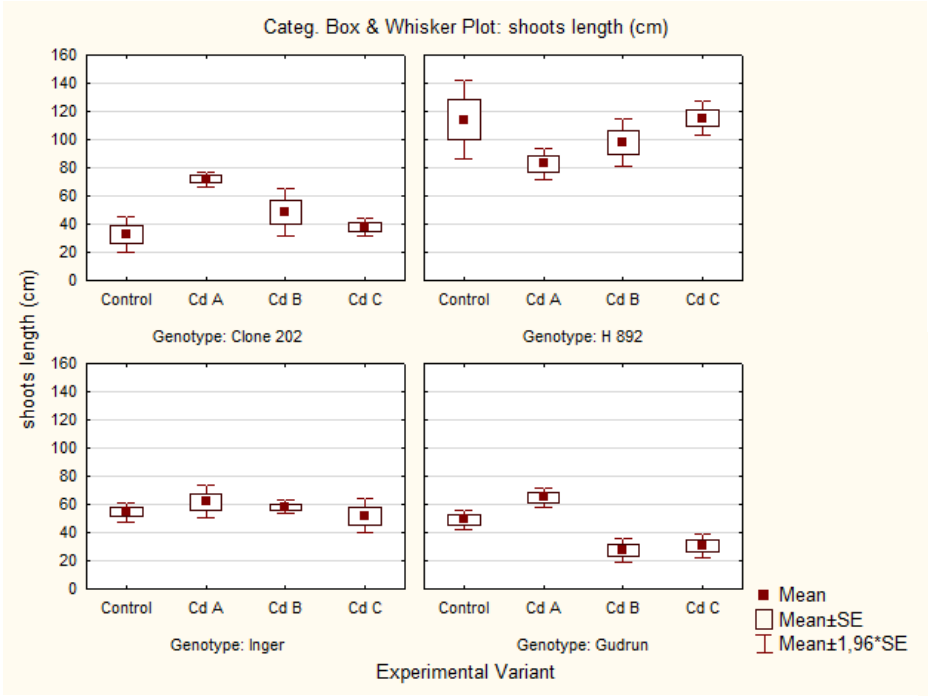
Analysis of Variance (exp salik butasi MG Cd) Marked effects are significant at p < ,05000

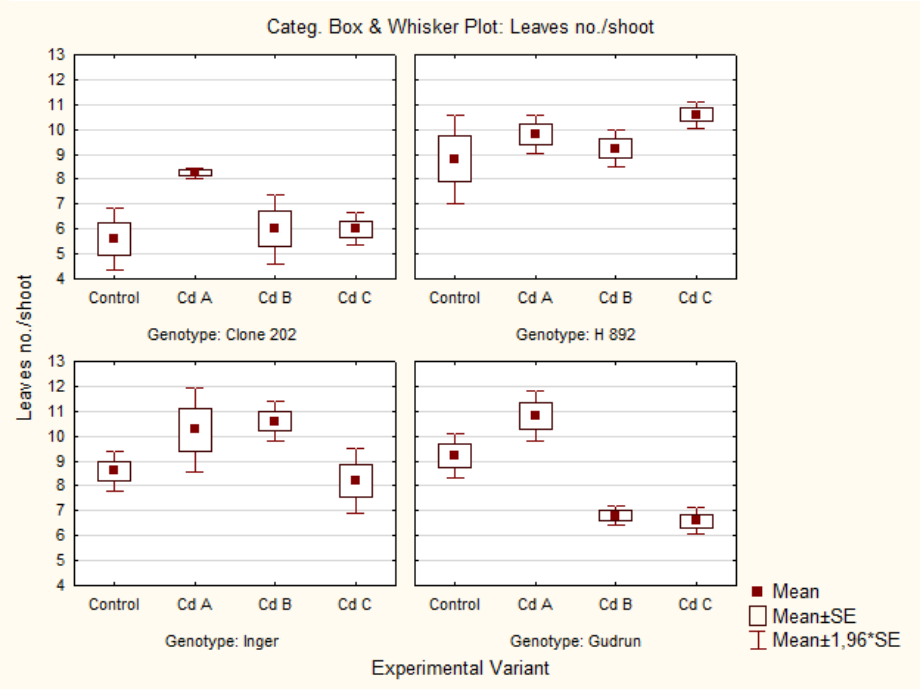
	SS - Effect	df - Effect	MS - Effect	SS - Error	df - Error	MS - Error	F	p
% active buds	6274,347	3	2091,449	151522,6	241	628,725	3,326495	0,020358
no. shoots/bud	2,438	3	0,813	73,7	241	0,306	2,657760	0,048986
shoots length (cm)	5299,173	3	1766,391	311693,4	241	1293,334	1,365766	0,253871
Leaves no./shoot	142,598	3	47,533	1534,3	241	6,366	7,466266	0,000084





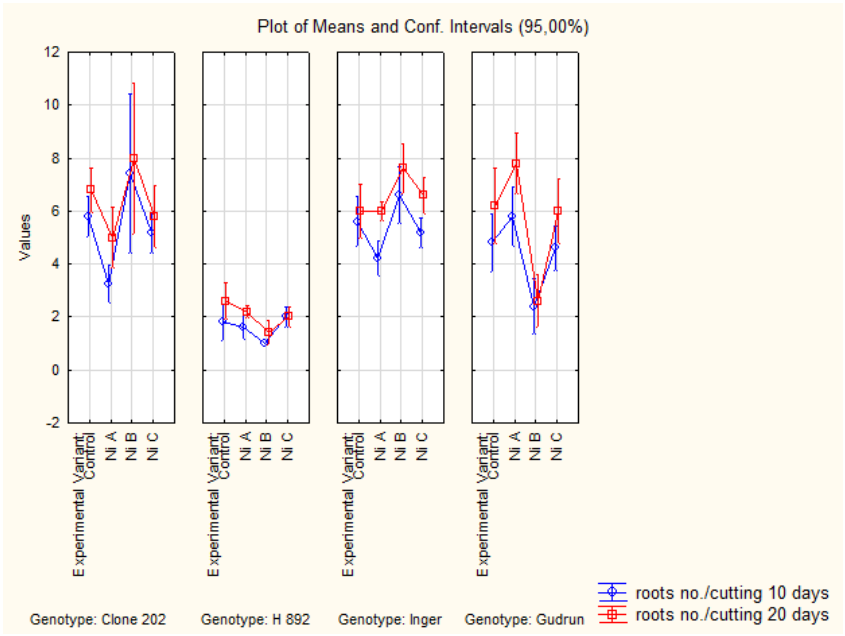
Shoots growth and foliar organogenesis – Cd





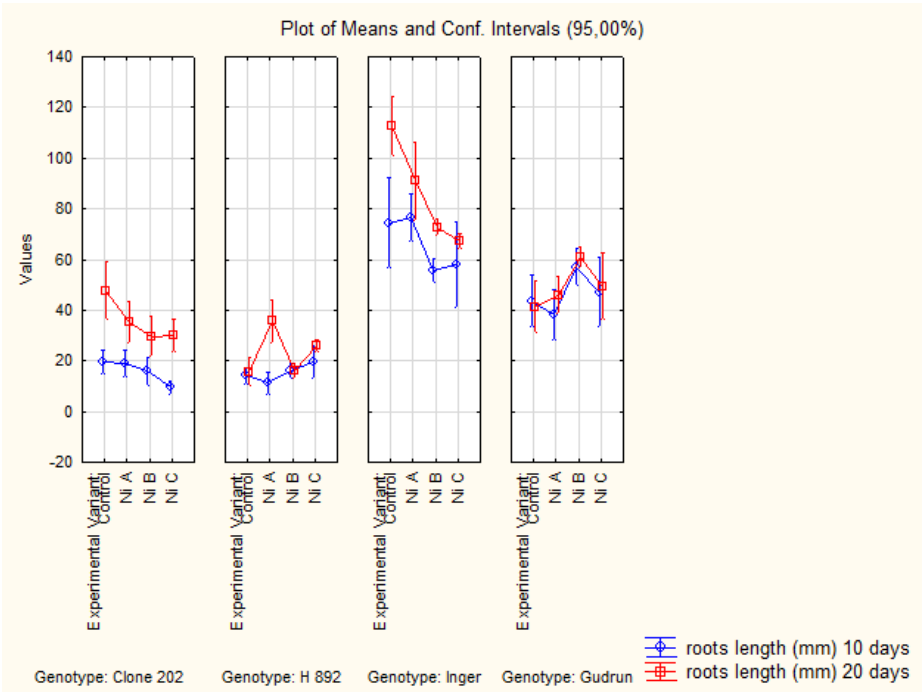
Cd (1-3ppm/l) stimulate foliar organogenesis, in most genotypes.

Rhysogenesis process
Ni



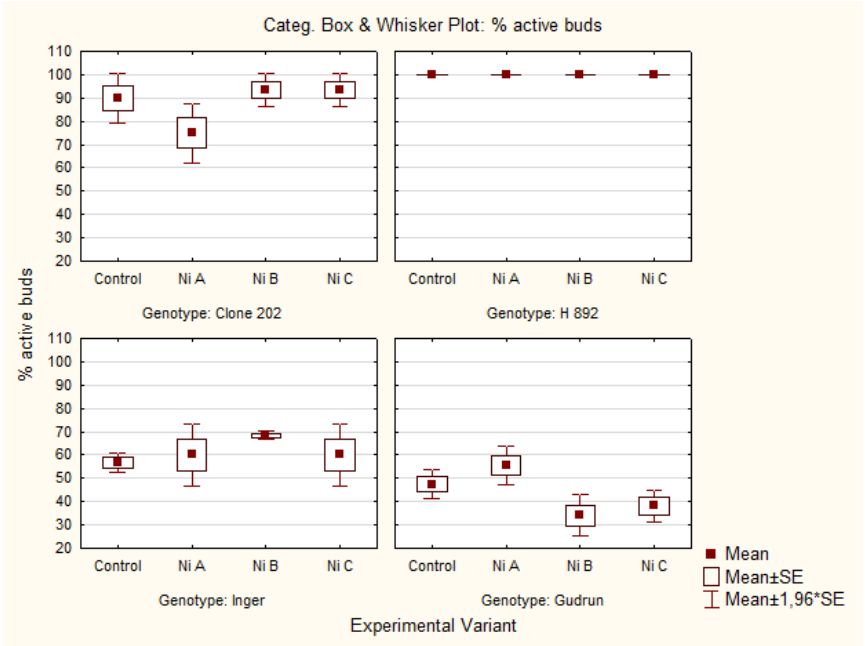
Analysis of Variance (exp sallx Ni) Marked effects are significant at p < ,05000

	SS - Effect	df - Effect	MS - Effect	SS - Error	df - Error	MS - Error	F	p
roots no./cutting 10 days	21,85	3	7,28	1690,6	237	7,1333	1,02110	0,383971
roots no./cutting 20 days	8,18	3	2,73	2082,5	237	8,7870	0,31021	0,818001
roots length (mm) 10 days	583,17	3	194,39	180767,8	237	762,7331	0,25486	0,857830
roots length (mm) 20 days	5234,98	3	1744,99	207765,6	237	876,6480	1,99053	0,116072



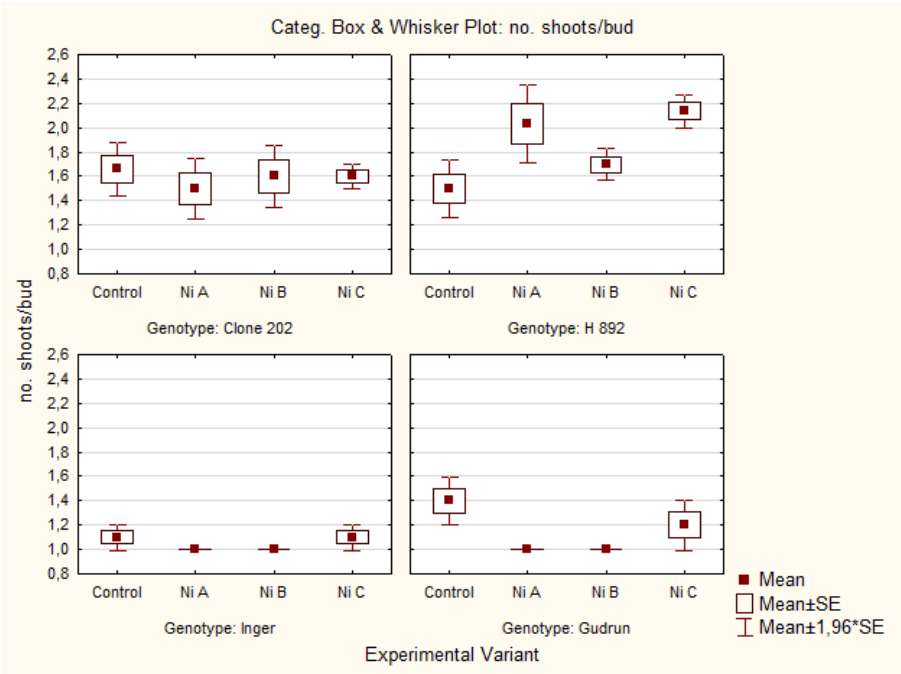
Clone 202 and Inger are most sensitive to Ni.

Shooting process
Ni

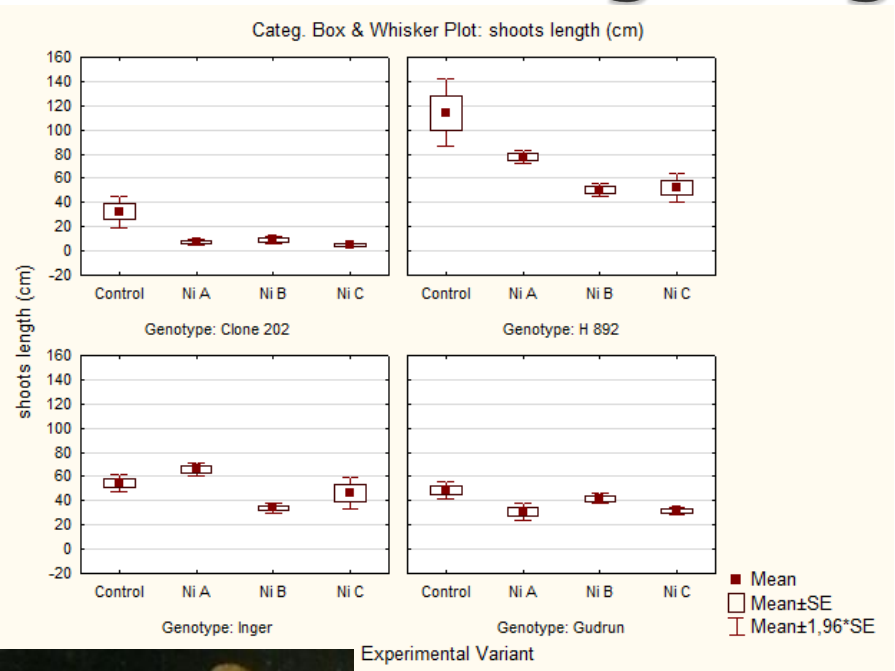


Analysis of Variance (exp salix Ni) Marked effects are significant at p < ,05000

	SS - Effect	df - Effect	MS - Effect	SS - Error	df - Error	MS - Error	F	p
% active buds	62,26	3	20,75	17949,3	237	757,3597	0,02740	0,993866
no. shoots/bud	1,05	3	0,35	57,0	237	0,2403	1,45903	0,226417
shoots length (cm)	33183,53	3	11061,18	225057,6	237	949,6100	11,64813	0,000000
Leaves no./shoot	224,41	3	74,80	1838,7	237	7,7582	9,64201	0,000005



Shoots growth and foliar organogenesis – Ni



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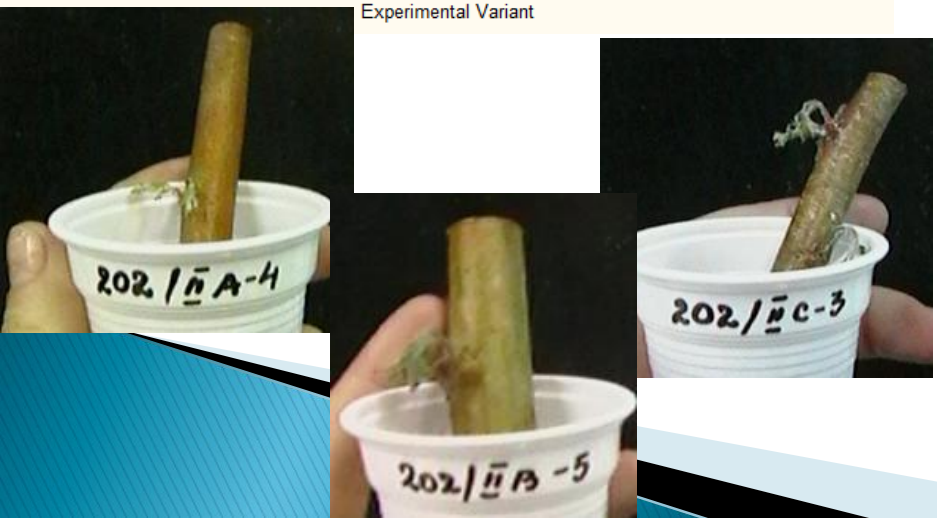
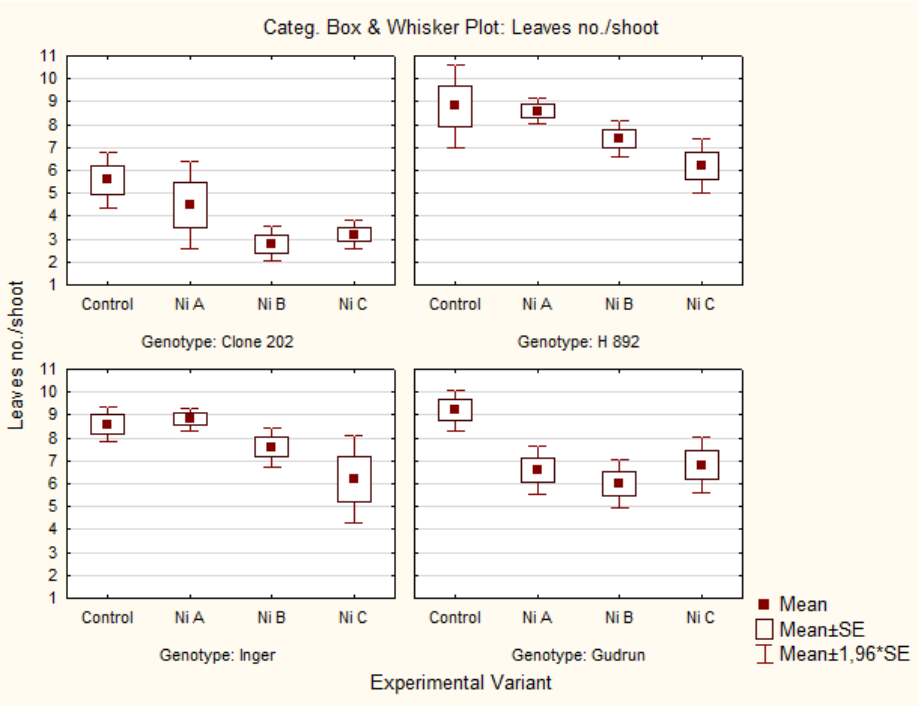
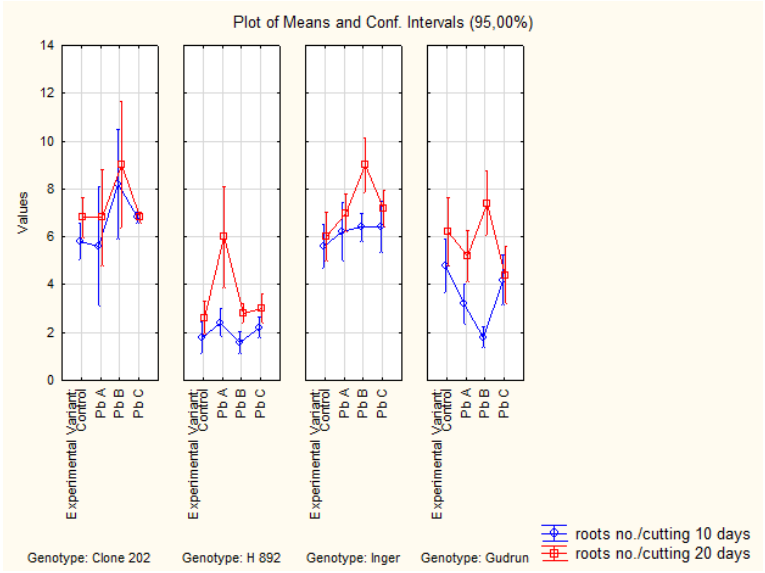
Ni inhibits shoots elongation, as well as the foliar organogenesis



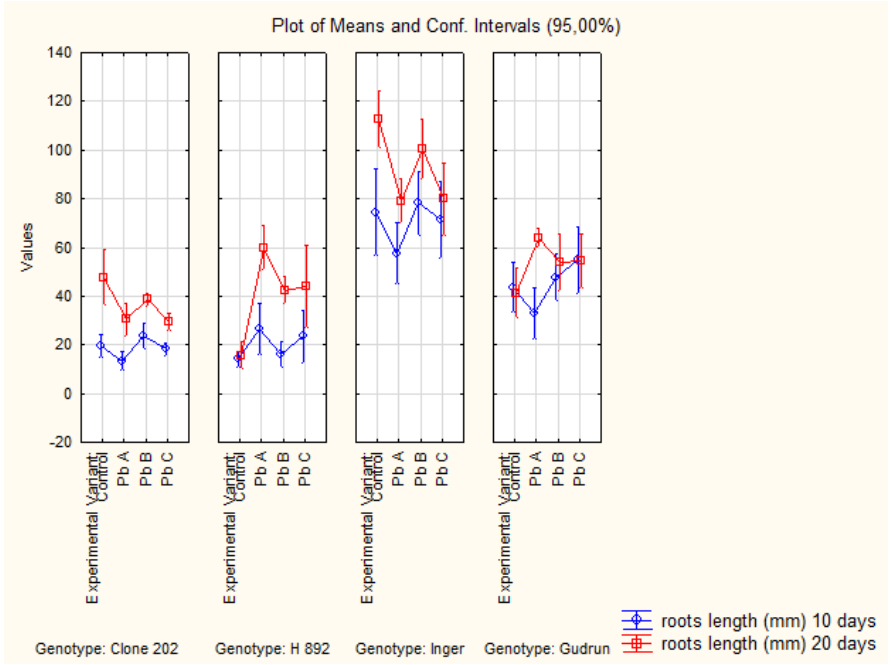


Rhysogenesis process
Pb



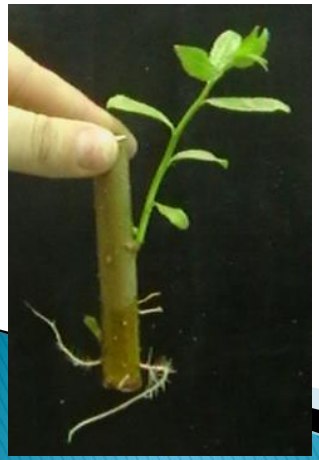
Analysis of Variance (exp sallx Pb) Marked effects are significant at p < ,05000

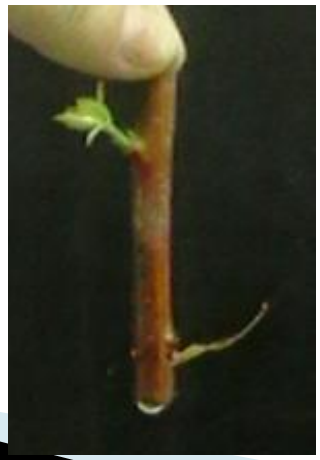
	SS - Effect	df - Effect	MS - Effect	SS - Error	df - Error	MS - Error	F	p
roots no./cutting 10 days	10,01	3	3,338	1933,1	236	8,191	0,407465	0,747777
roots no./cutting 20 days	116,81	3	38,938	2004,2	236	8,492	4,585111	0,003851
roots length (mm) 10 days	3259,35	3	1086,451	192692,6	236	816,494	1,330629	0,265065
roots length (mm) 20 days	1977,83	3	659,277	223272,6	236	946,070	0,696858	0,554797



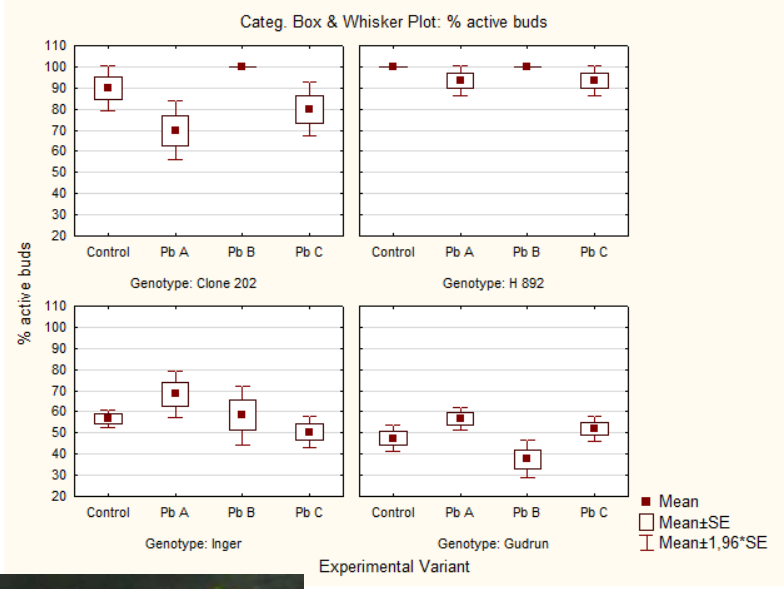
C 202 Control

Pb 450 ppm/l





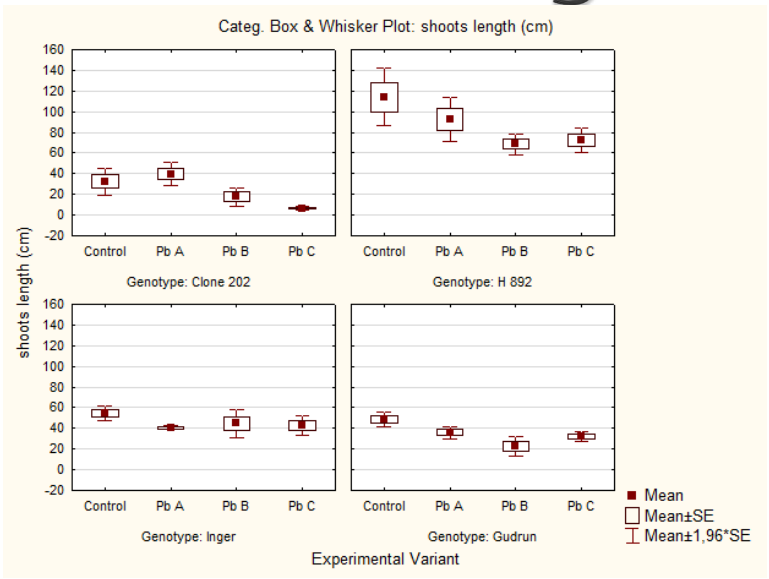
Shooting process
Pb



Analysis of Variance (exp salik MG Pb) Marked effects are significant at p < ,05000

	SS - Effect	df - Effect	MS - Effect	SS - Error	df - Error	MS - Error	F	p
% active buds	934,14	3	311,380	166560,7	236	705,766	0,441194	0,723756
no. shoots/bud	3,05	3	1,015	59,6	236	0,253	4,019907	0,008152
shoots length (cm)	24964,09	3	8321,364	276481,9	236	1171,533	7,102968	0,000137
Leaves no./shoot	183,60	3	61,200	1897,8	236	8,042	7,610496	0,000070

Shoots growth and foliar organogenesis – Pb



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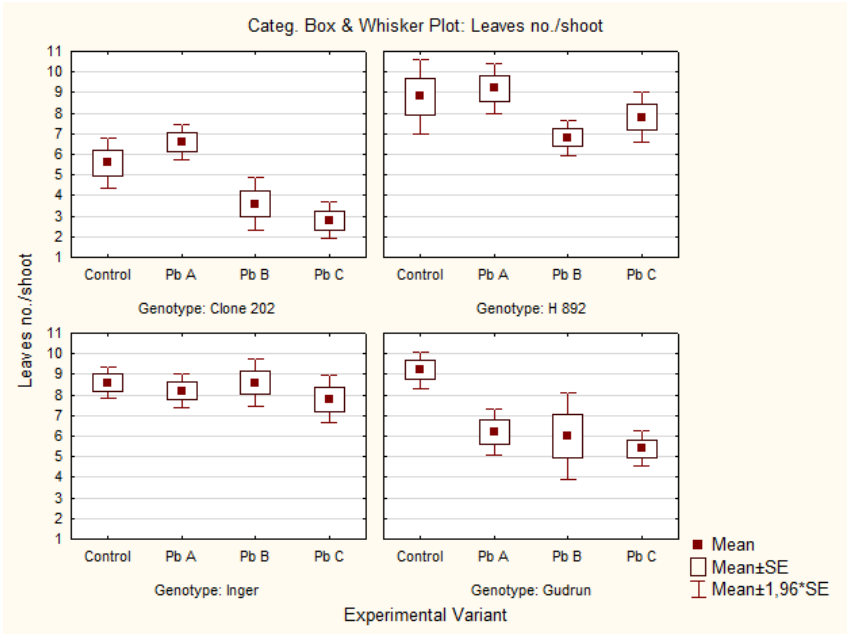
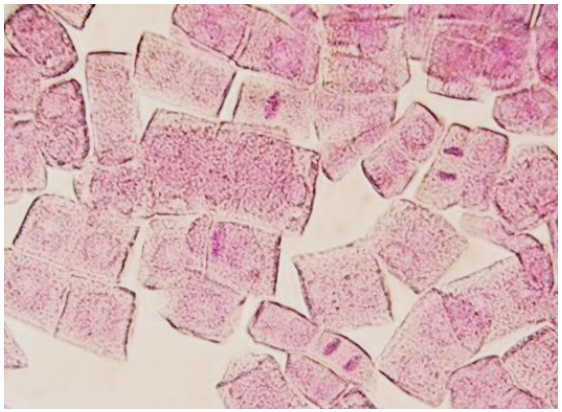
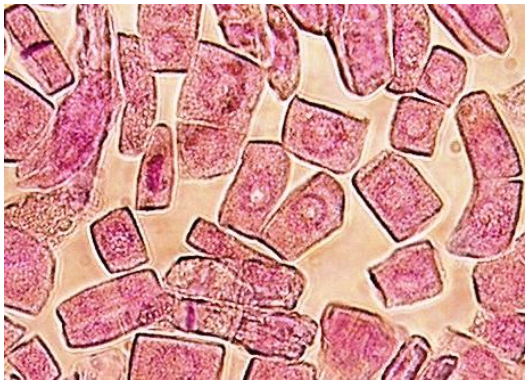
H890 Pb 50ppm/l 450 ppm/l

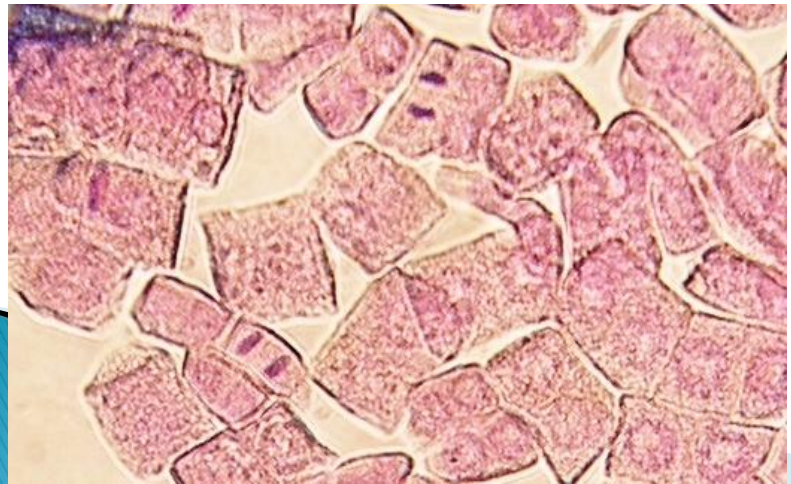


Cytological investigations

Studies on cell division, can explain the effects of different types of experimental factors on the meristems , organogenesis and growth processes.







S. alba 2n = 76
S. viminalis 2n=38

Experimental variant		Mitotic index (%)
Metal	Concentration	*Salix alba*
	ppm	H 892
Control	0	5.07
Cd-24 h	1	5.82
	3	5.90
	6	5.75
Ni - 24 h	50	4.17
	150	4.18
	450	2.29
Pb – 24 h	50	5.13
	150	4.54
	450	3.39

CONCLUSIONS

- There are significant differences, regarding the developmental behaviour among the genotypes

- CLONE 202 – tollerant; Ni > Pb > Cd
- H 892– rezistant ; Cd > Ni > Pb

- INGER– tolerant Ni > Cd > Pb
- GUDRUN– sensitive; Ni > Pb > Cd

- The cell division is normal in the first cycle; in the second cycle (48 h) Ni and Pb, as well as high concentration of Cd , are binding to the spindle fibers and produce its destruction(C–mitosis), or induce chromosomes agglutination and the division is stopped

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Thank you for attention!

QUESTIONS?