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Characteristics of physical properties of genetically modified potatoes. II. Mechanical properties of tubers

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A b s t r a c t. An investigation into the influence of genetic modification on potato tubers should include physical characteristics affecting their technological usability. Potato tubers of 15 clones of cultivar Irga transformed with viral genome sequences in order to improve their resistance to a necrotic strain of potato virus Y (PVY^N) were examined. The mechanical properties of potato tubers were determined using a compression test and expressed by fracture stress, F(kPa); fracture strain, D (%); and Young modulus, E (kPa).

The variability of F, D, and E for particular clones measured by standard deviations was extremely high. However, the influence of the modification type and breeding conditions appeared to be statistically significant but irregular during the three-year period of cultivation. While the effect of cultivation year was unmistakable, the order of F, D, and E values for the particular clones was not constant in the successive years. Results of detailed statistical analysis (variance and discrimination analyses) confirmed also a lack of stable tendencies in average values of the mechanical parameters for both groups and subgroups of clones representing the same modification model. No repeatable and statistically significant differences in F, D, and E were found, neither for the genetically modified nor for the control tubers. It can be concluded that the mechanical properties of potato tubers of cultivar Irga were not permanently affected by the type of genetic modification used.

K e y w o r d s: genetically modified potatoes, tuber, mechanical properties

INTRODUCTION

The mechanical properties and rheological behaviour of raw potato tubers have been widely examined for both scientific and technological purposes. The standard parameters and conditions of determination are, however, not commonly accepted. Various methods, such as uniaxial compression (Thybo and van den Berg, 2002, Blahovec, 2001), tensile test (Verlinden *et al.*, 2000), penetration tests (Anzaldua-Morales *et al.*, 1992), puncture test (Ranganna *et al.*, 1998) and numerous variants of small deformation tests (Laza *et al.*, 2001) have been proposed for the evaluation of the mechanical/textural parameters of raw and treated potato tubers.

During harvest and transport, potato tubers are exposed to impact damage which ranges from internal black spot bruising through shatter bruising and finally tissue cracking (Mathew and Hyde, 1997). The extent of damage can be primarily attributed to physiological changes which affect structural components (Thybo *et al.*, 1998), tissue turgor and temperature affecting failure properties of tubers (Alvarez and Canet, 2002; Bajema *et al.*, 1998). Thus, the recognition of the mechanical characteristics of potato tubers permits improvement of harvesting and handling equipment and operations aimed at reducing economic losses. Mechanical properties of tubers (as a measure of texture), beside the size and shape, are important factors of cultivar classification for such technological destinations as chips, French fry strips, and cooked potato (Dale and Mackay, 1994). The mechanical/rheological parameters of tubers obtained using various instrumental methods mentioned above have also been widely studied for development of algorithm of instrumental methods for the prediction of sensory attributes (Laza *et al.*, 2001; Solomon and Jindal, 2003; Thybo and van den Berg, 2002; Truong *et al.*, 1997).

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On the other hand, mathematical modelling of the mechanical properties of turgid plant tissue could enable an understanding of why different fruits and vegetables have different mechanical properties and how these properties can be changed (Blahovec, 2001; Hepworth, 2000).

Then, the mechanical properties of potato tubers play an important role in their physical characteristics and the investigation into the effect of genetic modification on potatoes against insect pests must contain also the mechanical characteristics of tubers.

MATERIALS AND METHODS

Material

Potato tubers of cultivar Irga were transformed with viral genome sequences in order to improve their resistance to a necrotic strain of potato virus Y (PVY^N). The transgenic clones were produced at the Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw. The genetically modified (GM) potatoes were bred and collected at the Plant Breeding and Acclimatization Institute, Młochów Research Centre (for details, see Sadowska *et al*., 2004). Samples of all the examined potatoes were stored under the same conditions and tested at the same time after harvest.

Methods

Compression test

For the elimination of possible effect of tuber size on the mechanical characteristics of potatoes (Anzaldua-Morales *et al*., 1992), six tubers of similar size and weight were selected from 1 kg sample. From each of the tubers, 3 cylindrical samples of 16 mm diameter were cut using a cork borer perpendicularly to the tuber length axis. Three pieces of 10 mm height were cut, in the central region and close to each end of each sample. Such preparation of the samples, with the diameter-to-height proportion proper for uniaxial compression from all the morphological parts of tubers of different microstructure, guarantied the obtainment of representative data.

The compression tests were made using the plate/anvil device of Instron 1011. The crosshead travel speed was 20 mm/min. The mechanical characteristics was expressed by fracture stress F, kPa, fracture strain D, %, and Young modulus measured in the elastic region of compression, E, kPa.

Dry matter content of tubers was determined according to AOAC method No. 984.25.

The statistical analysis of results was carried out with the Statistica v. 5 Software (General Convention and Statistics, StatSoft, USA, 1995).

RESULTS

Mean dry matter content (DM) of modified potatoes ranged from 19.72 to 23.02%, from 19.63 to 23.23% and from 20.69 to 24.38% for tubers from 2000, 2001 and 2002, respectively (Fig. 1). In 2000 and 2001, the values of dry matter content of tubers of the control cultivar Irga and Irga wt. were lower, and in 2002 almost the same as those of GM potatoes. The lack of correlation between DM and fracture stress and strain, and weak but statistically significant correlation between DM and elasticity modulus were found

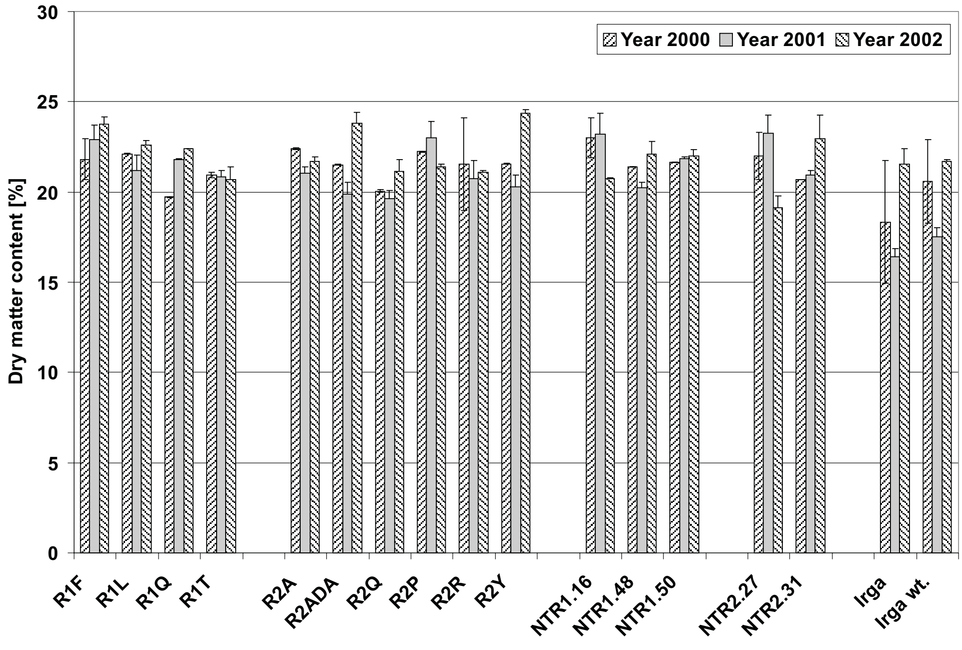

Fig. 1. Content of dry matter for GM potato tubers.

($R = 0.4932$ at $p < 0.05$). Though elasticity modulus is known to be closely connected with potato tissue turgor, Alvarez and Canet (2002) reported quite a low total decrease of the Young modulus during three months. These relationships confirmed that irregular changes of DM were almost always too small to be effective in influencing the mechanical properties of tubers.

In 2000, the highest values of fracture stress, F, fracture strain, D, and elasticity modulus, E, were reported for NTR1.16 and R2ADA (1.40 and 1.34 MPa), R1L and R1Q (55.6 and 56.3%), and NTR1.16 and R1L (5.64 and 5.29 MPa), respectively. The lowest values of F, D, and E were determined for NTR1.50 and R2Y (1.13 MPa for both clones), NTR2.27 and NTR2.31 (44.7 and 45.3%), and R2Y and R2Q (4.62 and 4.77 MPa), respectively (Figs 2-4). Yet, the respective values of the above-mentioned parameters for samples of the control group *ie* Irga and Irga w.t. were: for F - 1.17, 1.12 MPa, for D - 45.2 and 43%, and 4.66 and 4.65 MPa. Thus,

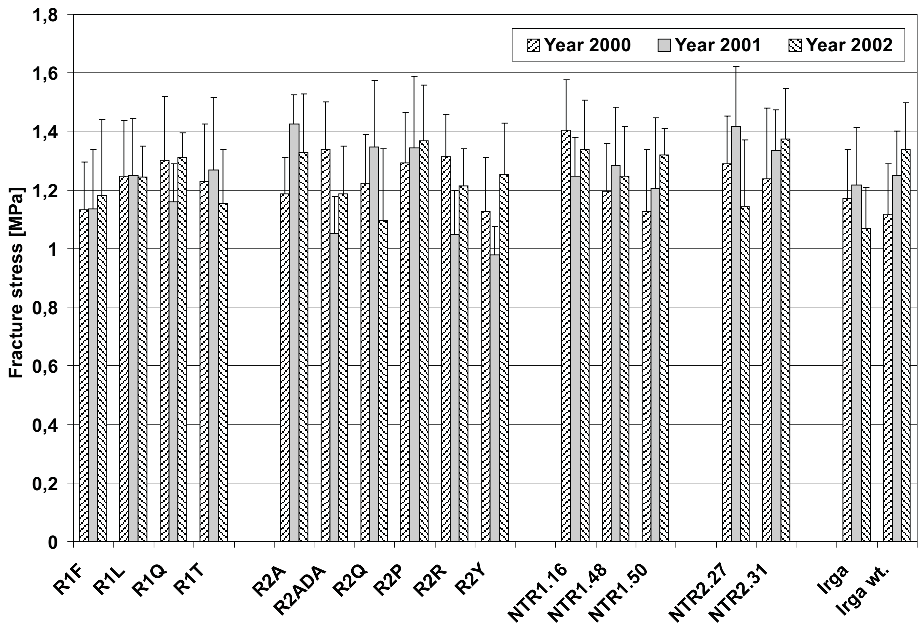

Fig. 2. Fracture stress for GM potato tubers.

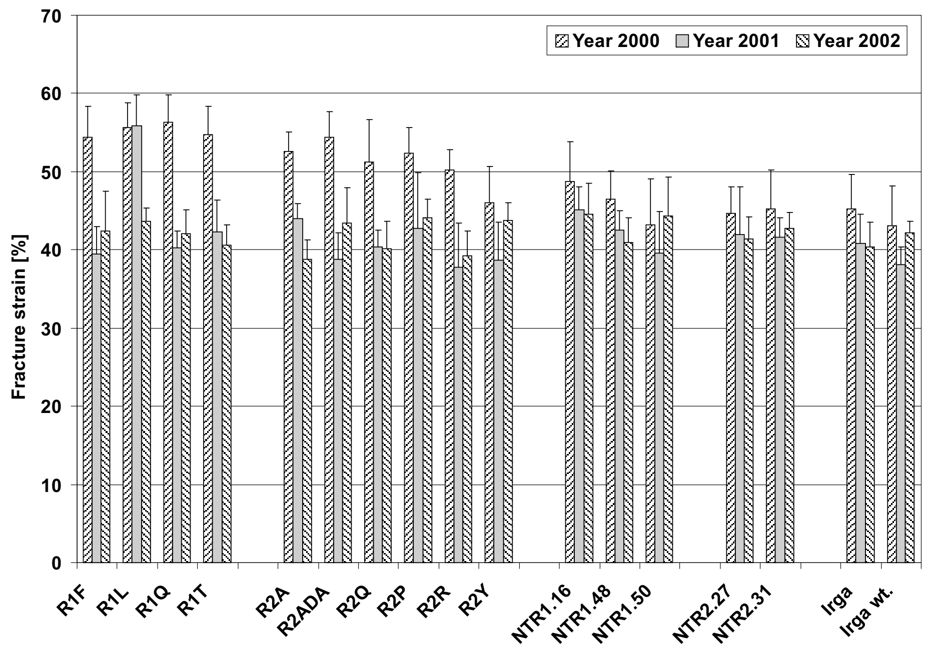

Fig. 3. Fracture strain for GM potato tubers.

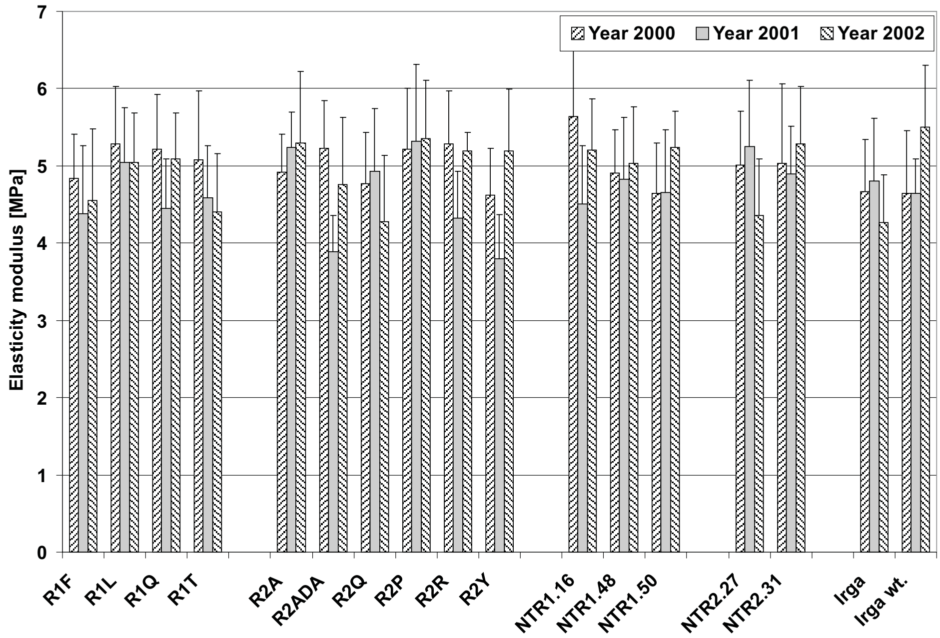

Fig. 4. Elasticity modulus of GM potato tubers.

F and E values of the control tubers were clearly lower than those for modified clones, while D values were in low range of all presented data. It could be probably one of few exceptions in which lower DM content resulted in lower mechanical parameters (average DM were 19.5 and 21.5% for control and modified tubers, respectively).

In 2001, the highest values of fracture and strain, and elasticity modulus were found for R2R and NTR2.27 (1.42 for both clones), R1L and NTR1.16 and R1L (45.1 and 45.8%), and NTR2.27 and R2Q (5.25 and 5.31 MPa), respectively. The lowest values of those parameters were determined for R2ADA and R2R (1.05 MPa for both clones), R2R and R2Y (37.8 and 38.7%), and R2Y and R2ADA (3.8 and 3.89 MPa), respectively (Figs 2-4). In that year, the respective values of mechanical parameters for Irga and Irga w.t. were 1.21 and 1.25 MPa, 40.8 and 38.1%, and 4.81 and 4.64 MPa. The F and E values for the control samples were similar to mean values for Is and IIs groups of modified clones, while D values were closer rather to D values of Ias and IIas groups.

In 2002, the highest values of fracture and strain, and elasticity modulus were found for R2P and NTR2.31 (1.4 for both clones), R2P and NTR1.16 (44.1 and 44.6%), and R2A and R2P (5.3 and 5.35 MPa), respectively. The lowest values of those parameters were determined for R2Q and NTR2.27 (1.10 and 1.14 MPa), R2A and R2R (38.8 and 39.3%), and R2Q and NTR2.27 (4.28 and 4.36 MPa), respectively (Figs 2-4). The respective values for Irga and Irga w.t. were 1.07 and 1.34 MPa, 40.4 and 42.2%, and 4.26 and 5.5 MPa. Only in that year a statistically significant difference between the mechanical characteristics of both samples of the control group was noted, which was caused by different manner of seed material preparation.

The data presented shows that the changes of mechanical parameters for particular clones were found to be irregular during the three years of cultivation. The mechanical properties of tubers of the control group did not remain at a constant level, either. In 2000, the mechanical properties of potatoes of the control group were lower than those of the modified clones, but in 2001 and 2002 the mechanical characteristics of the control tubers were similar to the characteristics of most of the clones. A confirmed high variability of mechanical parameters within a clone was found. Thybo and van den Berg (2002) also pointed out that samples of potatoes were characterized by large variation between replications for particular cultivars and even for tubers of the same cultivar. However, the obtained average data of fracture stress and strain were similar or slightly lower than data for non-treated tubers of other cultivars presented by Maté *et al.*, (1998) and Alvarez and Canet (2000). Alvarez and Canet (2000) found also that storage time did not affect significantly fracture force and energy from the compression test of potato tubers stored at 4°C and 85% relative humidity for even 140 days.

Despite the low differences between the maximum and minimum mean values for the examined clones at high values of standard deviations, the results of multifactorial variance analysis, Manova, showed statistically significant (at $p < 0.05$) influence of both the modification type and the year of cultivation on all the examined parameters of tuber mechanical properties. Similar results of Manova (except for stress) were obtained for data from the two first years in which seed samples were prepared identically. Interactions of factors were always statistically significant, which means that the influence of the first factor was different at different levels of the second factor, and for optimal conclusion particular results and not means of different levels should be compared (Volk, 1965).

Then, the influence of modification type was estimated for each year separately, using the Anova variance analysis. The results obtained again confirmed statistically significant differences for all the determined mechanical parameters at $p<0.05$ for each year. Also, typical correlation between fracture stress and elasticity modulus (correlation coefficients were 0.9283, 0.9276 and 0.9354 for 2000, 2001 and 2002 years, respectively) could be accepted as the next confirmation of different mechanical characteristics of the examined clones, despite significant variability of collected data. Yet, the expected correlation between fracture stress and strain was statistically significant only for tubers from 2001. Bajema *et al.* (1998) found that changes in strain rate did not affect failure stress, but failure strain was reduced dramatically with increasing strain rate. Then, they concluded that cell rupture pressure was not a function of strain rate and reduction of fracture strain suggested different fracture mechanisms compared with the standard viscoelastic material. Such positive answers in Anova analysis of data from each year did not allow a definite conclusion that genetic modifications used did not affect mechanical properties of potato tubers, although it was impossible to select clones of stable distinguished constant mechanical characteristics. Thus, variance analysis in subgroups corresponding to the modification type was supplemented by Duncan multiple range test (Table 1). In many cases, differences in the mechanical parameters of clone tubers in a subgroup were not significant. In other cases, the order of clones in uniform groups of Duncan's test was not repeated in particular years. Such an arrangement of results of this analysis suggested the effect of climatic and breeding conditions to be stronger than the modification influence.

The purpose of the next step of calculation was to look for stable tendencies in the mechanical properties for groups and subgroups of clones representing the same modification model. Mean values of the mechanical parameters for groups and subgroups are presented in Tables 1 and 2. Mean values of failure stress and elasticity modulus for Group II have always appeared, except for Irga w.t. in 2002, to be higher than those for Groups I and the control (Table 2). It has been also observed that tubers in both subgroups of antisense position *ie* Ias and IIas, were more mechanically resistant than tubers of the sense position subgroups *ie* Is and IIs, respectively (Table 3). For statistical estimation of differences between the mentioned groups, discriminant analysis was performed. However, the results of statistical analysis did not allow distinguishing subgroups of

T a b l e 1. Summary of results of Anova with Duncan's multiple range test for evaluation of differences in mechanical parameters of clone tubers in modification subgroups

Group	Year 2000	Year 2001	Year 2002
		Fracture stress	
1s	not significant	not significant	not significant
1as	(R2Y,R2A,R2Q) (R2P, R2R, R2ADA)*	(R2Y,R2ADA,R2R) (R2P, R2Q, R2A)	(R2A,R2R,R2Q)(R2ADA,R2Y,R2P)
2s	(NTR1.50, NTR.48)(NTR1.16)	not significant	not significant
2as	not significant	not significant	not significant
Control	not significant	not significant	(Irga w.t.) (Irga)
		Fracture strain	
1s	not significant	(R1F,R1Q,R1T)(R1L)	not significant
1as	(R2Y) (R2RR2Q) (R2A,R2P,R2ADA)	(R2R,R2Y,R2ADA)(R2Q,R2R,R2A)	(R2A,R2R,R2Q)(R2ADA,R2Y,R2P)
2s	(NTR1.50) (NTR.48,NTR1.16)	(NTR1.50)(NTR1.48,NTR1.16)	not significant
2as	not significant	not significant	not significant
Control	not significant		not significant
		Elasticity modulus	
1s	not significant	not significant	not significant
1as	(R2Y,R2Q,R2A)(R2P,R2ADA,R2R)	(R2Y,R2ADA,R2R)(R2Q,R2A,R2P)	not significant
2s	(NTR1.50) (NTR.48,NTR1.16)	not significant	not significant
2as	not significant	(Irga w.t.) (Irga)	not significant
Control	not significant	not significant	(Irga w.t.) (Irga)

*The uniform groups of clones are in brackets.

T a b l e 2. Characteristics of tuber mechanical resistance for modification groups

Group	Year	Failure stress (MPa)		Failure strain (%)		Elasticity modulus (MPa)	
		Mean	St. dev.	Mean	St. dev.	Mean	St. dev.
I	2000	1.24	0.184	48.8	4.64	5.04	0.707
	2001	1.20	0.221	42.1	6.38	4.61	0.823
	2002	1.23	0.194	41.8	3.64	4.90	0.861
II	2000	1.25	0.210	45.7	4.94	5.04	0.925
	2001	1.31	0.193	42.2	4.41	4.86	0.833
	2002	1.28	0.176	42.8	3.79	5.02	0.763
Irga	2000	1.17	0.165	45.2	4.42	4.66	0.671
	2001	1.21	0.176	41.2	3.32	4.71	0.725
	2002	1.07	0.139	40.4	3.09	4.27	0.614
Irga w.t.	2000	1.12	0.175	43.0	5.09	4.65	0.804
	2001	1.25	0.153	38.1	2.28	4.64	0.450
	2002	1.34	0.159	42.2	1.42	5.49	0.805

T a b l e 3. Characteristics of tuber mechanical resistance for modification subgroups

Group	Year	Fracture stress (MPa)		Fracture strain (%)		Elasticity modulus (MPa)	
		Mean	St. dev.	Mean	St. dev.	Mean	St. dev.
Is	2000	1.23	0.198	45.3	3.59	5.11	0.742
	2001	1.20	0.199	44.2	7.46	4.60	0.761
	2002	1.21	0.190	42.1	3.62	4.70	0.796
Ias	2000	1.25	0.174	51.1	4.54	5.00	0.682
	2001	1.21	0.238	40.4	4.84	4.61	0.877
	2002	1.24	0.199	41.6	3.67	5.02	0.887
IIs	2000	1.24	0.217	46.1	5.35	5.06	0.972
	2001	1.25	0.186	42.6	4.17	4.66	0.856
	2002	1.30	0.146	43.2	4.25	5.16	0.603
Iias	2000	1.26	0.202	44.9	4.17	5.02	0.857
	2001	1.38	0.179	41.8	4.72	5.08	0.764
	2002	1.24	0.228	41.9	2.52	4.73	0.999

statistically different mechanical properties (Fig. 5). Wilks coefficients *ie* the measure of the accuracy of group recognition, which ranges from 0 (ideal group discrimination) to 1 (lack of group discrimination), were 0.4318, 0.7698 and 0.8575 for 2000, 2001 and 2002, respectively, and confirmed non-sharp separation of groups of different genetic modifications. Independently of the cultivation year, the percentage of proper classification of cases in all the accepted subgroups was always very low (Table 4) and data for the control group were even classified as one group for the year 2000.

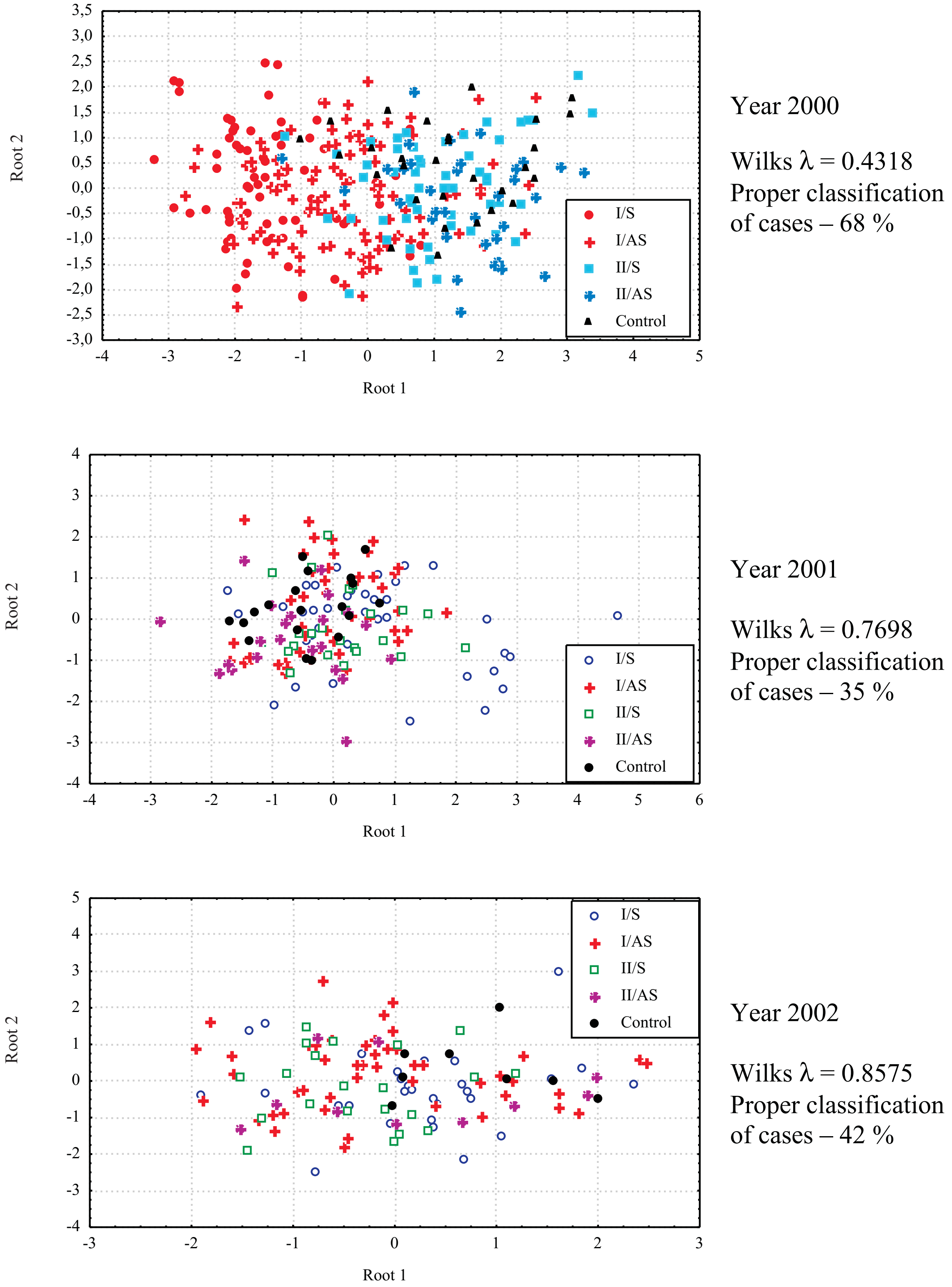

Fig. 5. Classification of modification type subgroups in successive year of cultivation.

T a b l e 4. Proper classification of cases (%) in discriminant analysis used for the estimation of variability of mechanical resistance of the examined GM clones

Group	Year		
	2000	2001	2002
Is	64	39	24
Ias	70	65	82
IIs	33	0	10
IIas	17	32	0
Control	23	0	0
Mean	51	35	42

CONCLUSIONS

1. The variability of all the parameters measured *ie* fracture stress and strain, and elasticity modulus for particular clones was extremely high, and the influence of modification type and breeding conditions appeared to be irregular during the three-year period of cultivation.

2. Stable tendencies in fracture stress and strain, and modulus of elasticity for groups and subgroups of clones representing the same modification model were not confirmed either.

3. Mechanical properties expressed by fracture stress and strain, and elasticity modulus of potato tubers of cultivar Irga were not permanently affected by the type of genetic modification used.

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