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Characteristics of physical properties of genetically modified potatoes. I. Mass and geometric properties of tubers**

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A b s t r a c t. 15 samples of potato tubers of Irga cultivar subgroups transformed with viral genome sequences (two modifications in the sense and antisense positions) in order to improve their resistance to a necrotic strain of potato virus were examined and compared to Irga tubers cultivated under normal and *in vitro* conditions during 3 successive years. The average weight and tuber weight distribution, and size and shape features (length, width and thickness, shape coefficient, and equivalent diameter) of tubers were measured using traditional and digital image methods. Tubers of the examined clones were classified as roundish/longish, except for longish/long tubers of two clones: R2P and R2Y. The variability of tuber shape, related to its size, for three modification subgroups was similar to constantly roundish Irga tubers, while tubers of only one subgroup were similar to Irga w.t. tubers, whose bigger tubers were more oblong. Then, it can be concluded that genetic modifications of Irga potatoes did not affect permanently the size and the shape of tubers, neither a for single clone nor for clone groups in modification subgroups. The year of cultivation and seed preparation were factors significantly affecting the weight, size, and shape of tubers of normal and genetically modified potatoes.

K e y w o r d s: genetic modification, potato, tuber, physical parameters

INTRODUCTION

Potatoes are an excellent source of carbohydrates, proteins, and bioactive compounds *ie* inhibitors of digestive enzymes, polyphenols, glycoalkaloids, vitamins and others. The consumption of potatoes varies from country to country, but takes a remarkable position in human diet (150 -400 g and more per capita per day). The major pest limiting the yield or quality of potato tubers are: Colorado potato beetle (CPB) and potato loaf roll virus (PLRV) and potato Y (PVY) viruses. The latter one is considered one of the damaging viruses which cause economically significant yield reduction, by as much as 80%. While the effectiveness of insecticides used to prevent undesired changes of the quality of tubers and yield losses varies due to pesticidal characteristics limitations, genetical transformation seems to be the most effective method introducing the required resistance.

Variation of potato tuber size is usually very high, even for a single plant, because of the differing development of particular tubers during harvest. Additionally, tuber size of potato cultivars depends on many external factors, such as climatic conditions and agricultural treatment (Cepl and Vokal, 1996; Wurr *et al.*, 1997). For a long time, tuber size has been recognised to strongly influence the distribution of dry mass and other basic constituents, especially starch (Burton, 1966; Dale and Mackay, 1994; Rastovski *et al.*, 1981). Baritelle and Hyde (1999) noted that tuber size influenced the resistance of potato tissue to failure and Cmunt (1997) found also that tuber resistance to mechanical damage during harvest increased with increasing weight of tubers. On the other hand, food industry demands potato cultivars for special uses *ie* for chips or French fries of characteristic shape and size distribution. Through genetic selection and the use of special agricultural treatments, appropriate cultivars of desired geometric parameters have been bred. Thus, remembering the above-mentioned restrictions, changes of tuber geometry can be considered as variations of characteristic features of a cultivar.

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Flis and Zimnoch-Guzowska (2000) studied the effect of different constructs and consequently groups of clones on the variability of selected measures of potato plant and tubers and noted some differences between control and transgenic plants (plant appearance, plant vigour, days to foliage senescence, eye depth, secondary growth of tubers, tuber shape and weight). Yet, the size and shape of potato tubers play an important role in the design of equipment for harvest and technological processing, and studies on the variability of these features of GMO potatoes should be consequently extended.

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 first group (Group I) of constructs (R) introduced into the genome of cv. Irga contained a truncated gene coding PVY^N polymerase (Nib) (GenBank Acc. No. D00441) in the sense and antisense orientation. The second group (Group II) of clones (NTR) contained a fragment of cDNA corresponding to 184 nucleotides of the 5'-end of PVY of PVY^NWi isolate (GenBank Acc. No. Z70238) which were also introduced into potato genotype in the sense and antisense orientation. These two groups of transgenic clones contained the following subgroups:

- R1 with a truncated gene coding PVY^N polymerase in the sense orientation (subgroup Is - 4 clones), and R2 with the same viral gene in the antisense orientation (subgroup Ias - 6 clones);

- NTR1 with introduced fragment of cDNA of PVY of PVY^NWi isolate in the sense orientation (subgroup IIs - 3 clones), and NTR2 with the same sequence introduced in the antisense orientation (subgroup IIas - 2 clones).

The transformation process, selection and identification of transformed plants from cv. Irga were described by Chachulska *et al*. (1997).

Two types of non-transgenic control tubers were also examined *ie* 1/ obtained from normally planted cv. Irga (Irga), and 2/ obtained from in vitro cultures (Irga w.t.).

The potatoes were bred and collected at the Plant Breeding and Acclimatisation Institute, Młochów Research Center. In 2000 and 2002 all modified clones and control Irga w.t. were propagated by successive passages of minitubers obtained *in vitro* (Flis and Zimoch-Guzowska, 2000), but in 2002 minitubers obtained directly from *in vitro* plants were used in a field experiment. The weight of potato samples ranged from 1000 to 1500 g while the number of tubers in a sample ranged from 18 to 63.

Methods

Tubers were weighed with the accuracy of ± 0.1 g using a laboratory scales 1600C (Medicat Ltd., Poland). Distribution of tuber weight was compared using ordered data collections in which, according to the weight of single tubers ranging from the smallest – 20 g and highest – 240 g, 13 classes were created with a step of 20 g.

The size and shape of tubers were determined: 1/ traditionally, and 2/ using digital image analysis method.

1/ The size of tubers was expressed by length and width which were measured using a slide calliper with the accuracy of $\pm$ 0.1 mm. The stem end to bud end axis was accepted as the length. The axis perpendicular in the same plane to length axis measured in the broadest part of tuber was accepted as the width. The shape of tubers was expressed as length:width ratio (Winiger and Ludwig, 1974).

2/ Potato tubers were positioned in natural rest position on a glass table which was illuminated with 4 overhead light sources TUNGSRASOFT 100W (Tungsram, Hungary) symmetrically put in corners and dissipated white light from underneath for possible object shadow elimination. Black/White images with the size of 172 × 96 mm (768 ×576 pixels) were captured using CCD Elemis K45R camera (Elemis, Poland). Captured images were transferred to a Pentium class PC computer using Matrox Meteor frame grabber (Matrox, Canada) and stored as 8-bite TIFF files. The characteristics of the objects were taken using the Olympus Micro Image 4 software package (Olympus Europe, Germany). The sharpening of object edges was realised using automatic tresholding. Objects of an area less than 1 mm^2 were excluded automatically. Calibration of digital images was made by digitising a graph paper with a net of 1×1 mm, being captured in the some conditions. Each object was measured in three planes of length, width and thickness axes. Among typically measured geometric parameters of the objects, the following features were used in subsequent calculations: area, A (mm^2); maximum diameter, d_{max} (mm), and minimum diameter, d_{min} (mm). Maximum and minimum diameters corresponded closely to manually determined length and width of tubers, respectively.

The shape of tubers was additionally expressed as equivalent dimension, d_e, and average projected area *ie* mean of projected area measured , A_m, for three typical planes (length, width, and thickness – A_l, A_w, A_t) and calculated according to Mohsenin (1970) as follows:

$$A_m = (A_l + A_w + A_t) / 3.$$

Statistical analysis of the results was carried out with a Statistica ver.5 (StatSoft Inc., USA) software (1995) using also comments for methods used as described by Volk (1965).

RESULTS

Average weight of tubers of the examined GM potatoes is presented in Fig. 1. Although the average weight of tubers in 2000 and 2001 was typical for this cultivar, the presented values were always a little higher for tubers from 2001. On the contrary, tubers of potatoes harvested in 2002 were extremely small. It was probably caused by omitting successive passages of minitubers obtained *in vitro* obtained (Flis and Zimnoch-Guzowska, 2000). Data collected in 2002 were also included in statistical calculations, but the influence of the above-mentioned additional factor was considered permanently. Despite the high variability of tuber weight for both GM clones and control Irga cultivars (range of variation coefficients were 35.4-72.2%, 23.7-49.6%, 41.1-81.0% for 2000, 2001, and 2002, respectively), the results of Anova confirmed the statistically significant (at p=0.05) effect of the cultivation year. When Anova analysis was repeated for data of 2000 and 2001 only, the effect of the cultivation year appeared weaker, but still statistically significant. The results of double Anova analysis clearly pointed out an extremely high effect of preliminary operations (passaging of seed potato) on potato tuber size, which was also reported by Divis and Barta (2001) and Wurr *et al.* (2001).

Then, on account of high variability of this important tuber characteristic for all the samples examined, the distribution of tuber weight (TWD) was analysed. Tuber weight distributions for particular clones were compared with weight distributions of both control samples using Spearman correlation (Table 1). TWD of the control samples was similar only in 2000, which could indicate the effect of a different way of tuber propagation. Some of the

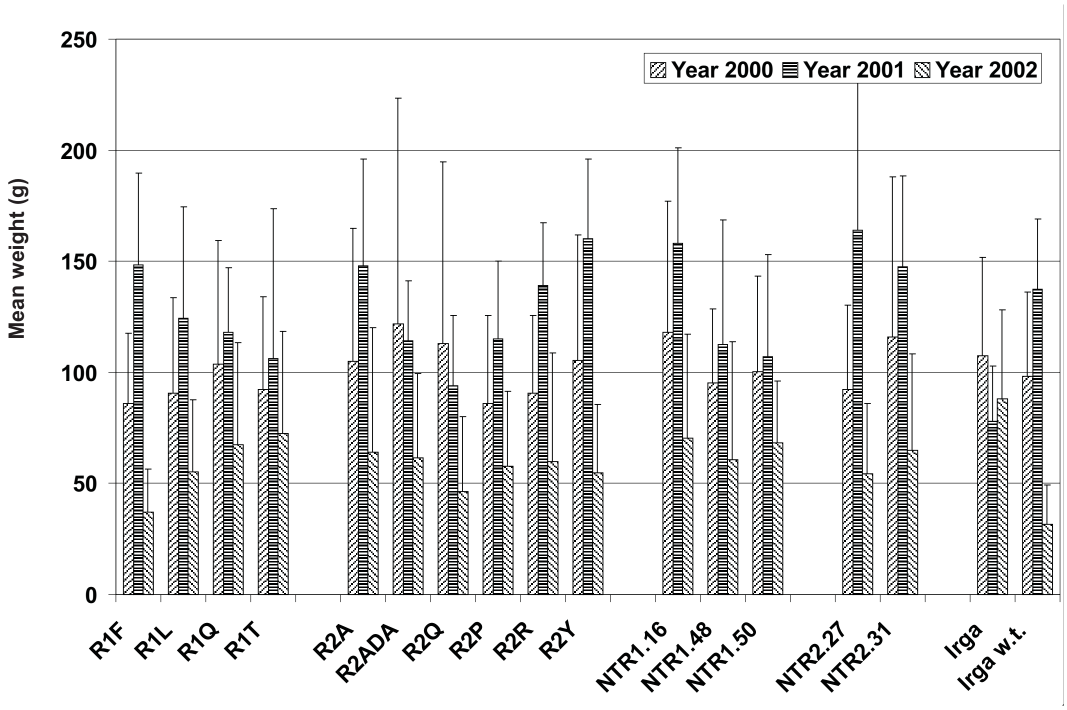

Fig. 1. Mean weight of GM potato tubers.

T a b l e 1. Statistical significance of Spearman correlation between tuber weight distributions of both control and GM clones

Samples	IRGA			IRGA w.t.		
	2000	2001	2002	2000	2001	2002
R1F	+	N	N	+	+	+
R1L	+	+	+	+	N	+
R1Q	N	N	N	+	+	+
R1T	+	N	+	+	N	N
R2A	N	N	N	N	N	+
R2ADA	+	+	+	+	N	+
R2Q	N	+	N	N	N	+
R2P	+	N	N	+	+	+
R2R	+	N	N	+	+	+
R2Y	N	N	+	+	+	+
NTR1.16	+	N	+	+	N	+
NTR1.48	+	+	N	+	N	+
NTR1.50	+	+	+	+	N	+
NTR2.27	+	+	+	+	N	+
NTR2.31	N	+	+	N	+	+
IRGA w.t.	+	N	N	-	-	-

+/ statistically significant, N/ not significant.

TDW of clones from different modification subgroups were always similar to Irga TDW (R1L, R2ADA, NTR1.50, NTR2.27L), while others were similar to Irga w.t. TWD (R1F, R1Q, R2P, R2R, R2Y). Similarity of the TWD of the remaining clones and the TWD of the control samples was found irregular in the successive years of cultivation (Table 1). As in 2001 and 2002 the TWD of Irga and Irga w.t. were not similar, the TWD of clones should be similar either to Irga or Irga w.t. However, only five clones (R1F, R1Q, R2P, R2R, and NTR2.31) appeared to agree with this logical expectation.

Yet, the obtained results of weight distribution variability for particular clones did not exclude the possibility of clearer relations between the mean distributions of tuber size for the modification subgroups and the control groups. Though in Fig. 2 the TWD for modification subgroups are presented in a more simplified but the same shape, calculations were also made for 13 range distribution. The results presented in Table 2 confirmed that resultant TWA of subgroups are similar to both control TWD in 2000. In the next cultivation years, they were

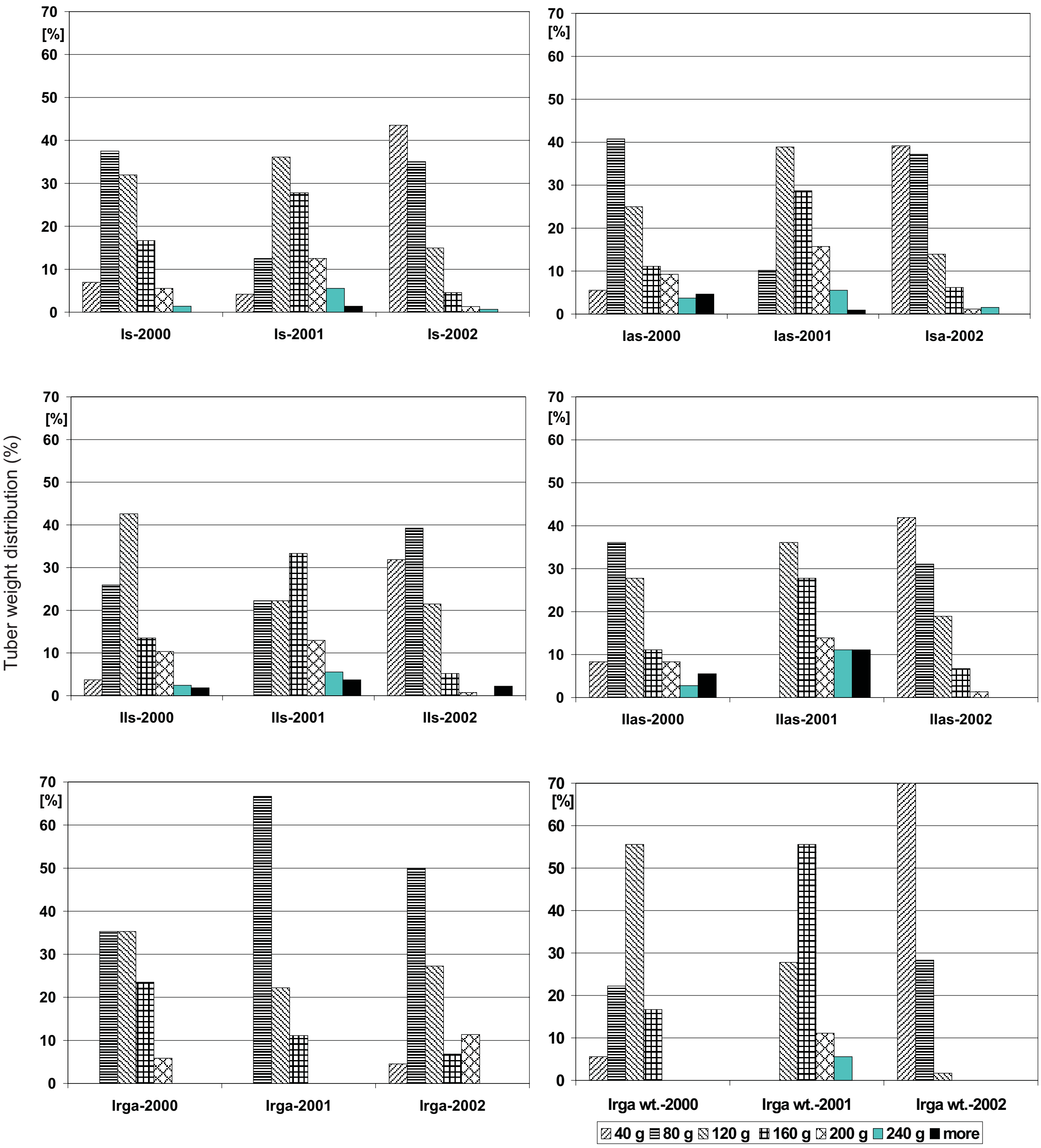

Fig. 2. Tuber weight distribution for modification groups of GM and control potatoes.

T a b l e 2. Statistical significance of Spearman correlation between tuber weight distributions of both control and modification subgroups of GM clones

Samples	IRGA			IRGA w.t.		
	2000	2001	2002	2000	2001	2002
Is	+	+	N	+	+	+
Ias	+	N	N	+	+	+
IIs	+	+	+	+	+	+
IIas	+	N	+	+	+	+
IRGA w.t.	+	N	N	-	-	-

+/ statistically significant, N/ not significant.

always similar to Irga w.t. TWD while Spearmann correlation between subgroups and control Irga changed irregularly. It is worth attention that all the distributions of tuber weight (except for group Is) from 2001 were characterised by the lack of the smallest tuber range, while in 2002 these ranges predominated in all the distributions.

Tuber density of the examined clones and control cultivars remained at a constant level (for clones 1061 kg m^{-3} on the average, for Irga 1071 kg m^{-3}, and for Irga w.t. 1065 kg m^{-3} at variation coefficient 4.5, 2.4, and 3.7%, respectively) whereas characteristic dimensions of single tubers *ie* length and width, were related to their weight (Fig. 3). The variation coefficients for these mean parameters were also high and ranged from 9 to 30% for the length and from 8 to 28% for the width of tubers.

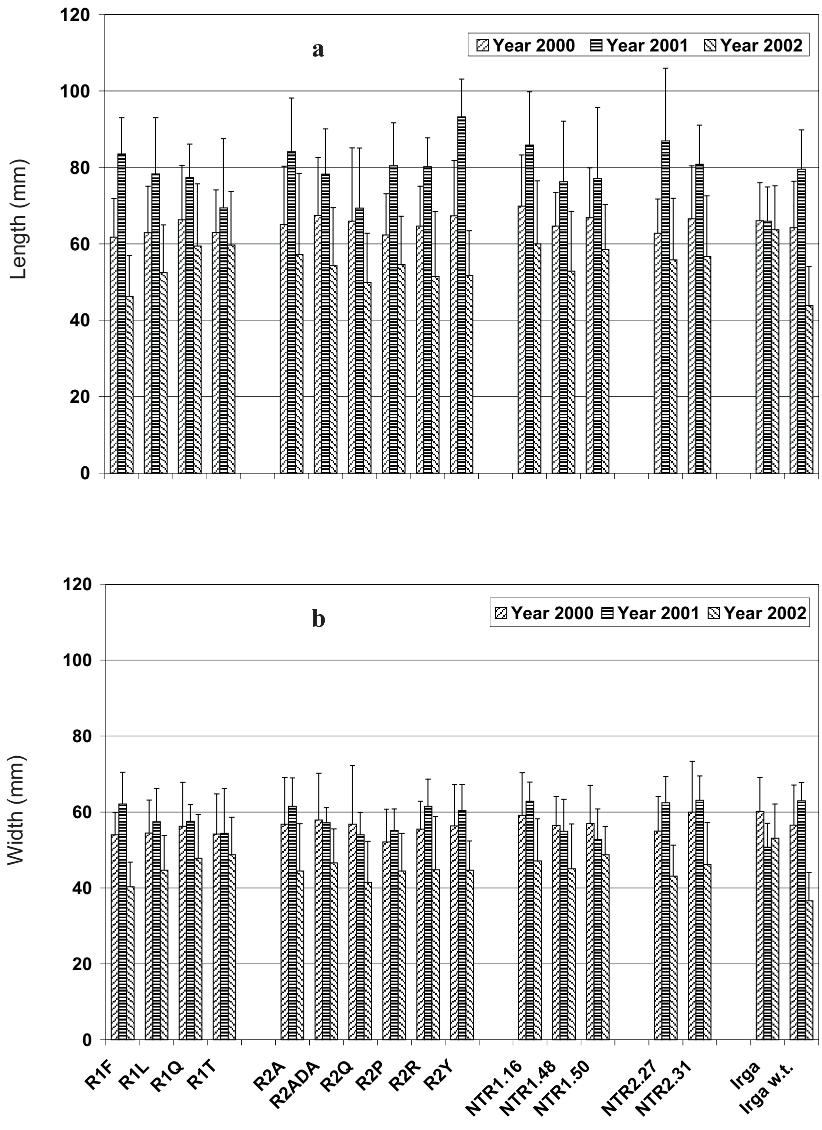

Fig. 3. Mean length (a) and width (b) of GM potato tubers.

The most often used index of tuber shape is the length:width ratio (shape coefficient), which appeared to be practically a reciprocal of a characteristic geometric parameter - sphericity (Fig. 4). Only two clones from 2002 (R2P and R2Y) can be classified in the longish/long class, which corresponds to shape coefficient values ranging from 1.40 to 1.69 (Winiger and Ludwig, 1974). All the remaining tubers were classified as roundish/longish (with respective range of shape coefficient 1.10 to 1.39). It is worth attention that tubers of all the examined samples collected in 2001 were characterised by higher shape coefficient values *ie* by more oblong shapes. Actually, the shape was more of a constant feature than the weight of single tubers, which was confirmed by the range of variation coefficients clearly narrower for the shape coefficient (from 6 to 12%) than for the mean weight of tubers (even up to 81%). A similar opinion was presented by Wurr e*t al.* (2001) who found that time of harvesting the seed crop did not affect the tuber shape nor the number of ground stems without interaction with tuber size. The mean equivalent diameter of tubers was decidedly the lowest in 2002, in which d_e for clones ranged from 3.95 to 4.90 cm and d_e for Irga and Irga w.t. were 5.31 and 3.71 cm, respectively. In 2000 and 2001, the ranges of d_e for clones were similar *ie* 5.31-6.56 and 5.47-6.58 cm, respectively, for Irga 5.11 and 5.31 cm, respectively, while for Irga w.t. it was extremely different at 6.25 and 3.71 cm, respectively. Variability of equivalent diameter seemed to be similar to tuber weight variability confirming a strong influence of the propagation system of potato seeds.

Additionally, for the description of the volume shape of tubers collected in 2000, a criterion area – Ac (cm^2) was determined. Based on the theory that the average projected

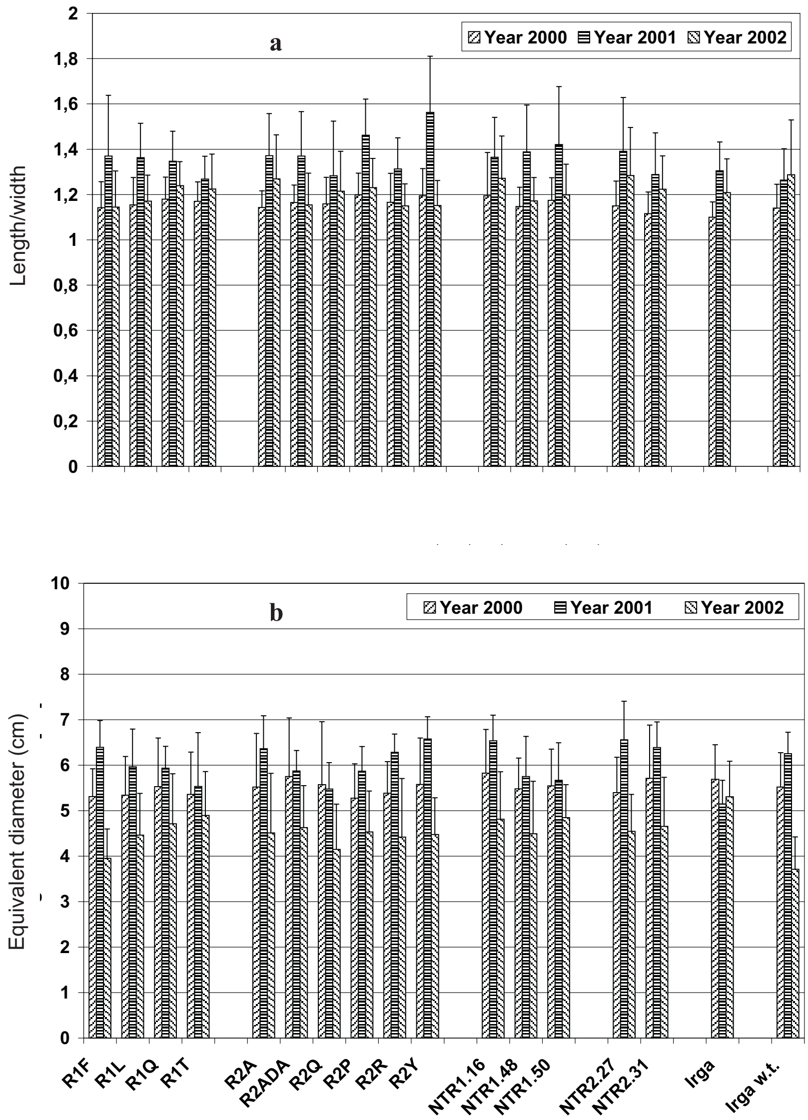

Fig. 4. Length/widht (a) and equivalent diameter (b) for GM potato tubers.

area of a convex body is a quarter of the surface area, Mohsenin (1970) presented the following equation:

$$Ac \leq K\, V^{\frac{2}{3}},$$

where: K is a measure of the degree of sphericity (for sphere K=1.21); values of K ranged from 1.18 to 1.37 showing, however, a differentiation of tubers shape; V is volume (cm^3).

Classic relationship between the criterion area and the volume of potato tubers (Fig. 5) allowed visual presentation of deviation of the examined tuber shapes from an ideal sphere. If all Irga tubers were roundish, then the bigger Irga w.t. tubers were more oblong. The relationships calculated for tubers of three modification subgroups (Ias, IIs, and IIas) were similar to that for Irga, while the relationship determined for the Is subgroup appeared more similar to that of Irga w.t.

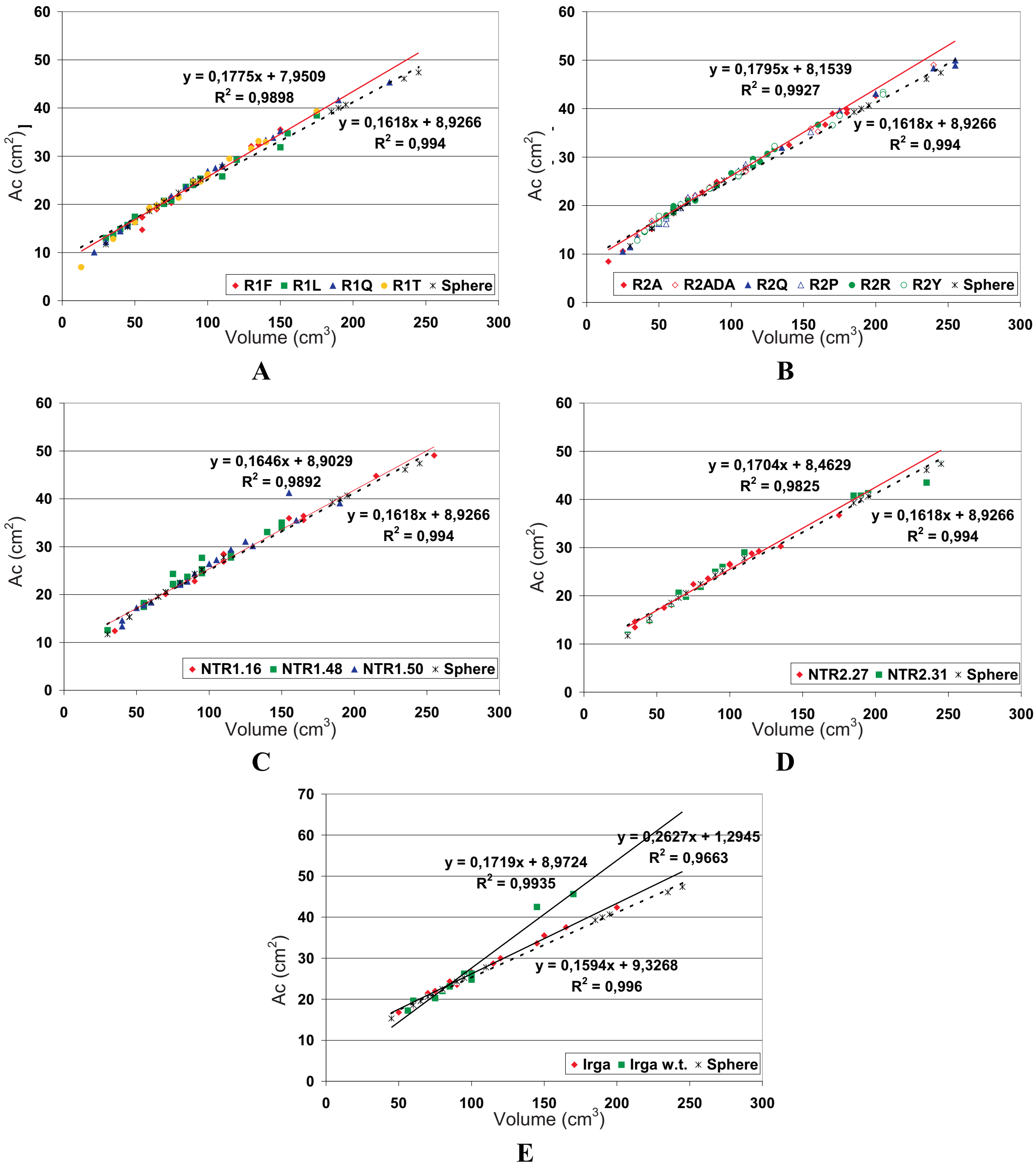

Fig. 5. Comparison of relationship between criterion area (Ac) and volume for potato tubers in modification groups (continuos line) and ideal sphere (dotted line) for Is (A), Ias (B), IIs (C), IIas (D), and control (E) subgroups.

CONCLUSIONS

1. Despite very high variability of all the measured parameters of the examined samples, the results of Anova analysis confirmed a statistically significant effect of the cultivation year and preliminary operations on tuber weight and shape.

2. Though the results of Anova confirmed also a statistically significant effect of modification type, still they pointed out various single clones and modification subgroups as different in the successive years of cultivation.

3. Comparison of the weight distributions for tubers of clones and Irga and Irga w.t. within the modification subgroups demonstrated their irregular changes in the successive years.

4. Tubers of clones and control samples were classified as roundish/longish, except for longish/long tubers of two clones: R2P and R2Y.

5. The variability of tuber shape, related to its size, for modification subgroups: Ias, IIs, and IIas was similar to constantly roundish Irga tubers, while the tubers of the Ia subgroup were similar to Irga w.t. tubers whose bigger tubers were more oblong.

6. The genetic modifications applied for Irga potatoes did not affect permanently the size and shape of tubers, neither for a single clone nor clone groups in modification subgroups, whereas the year of cultivation and seed preparation were the factors significantly affecting these properties for all the examined clones and the control cultivar.

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