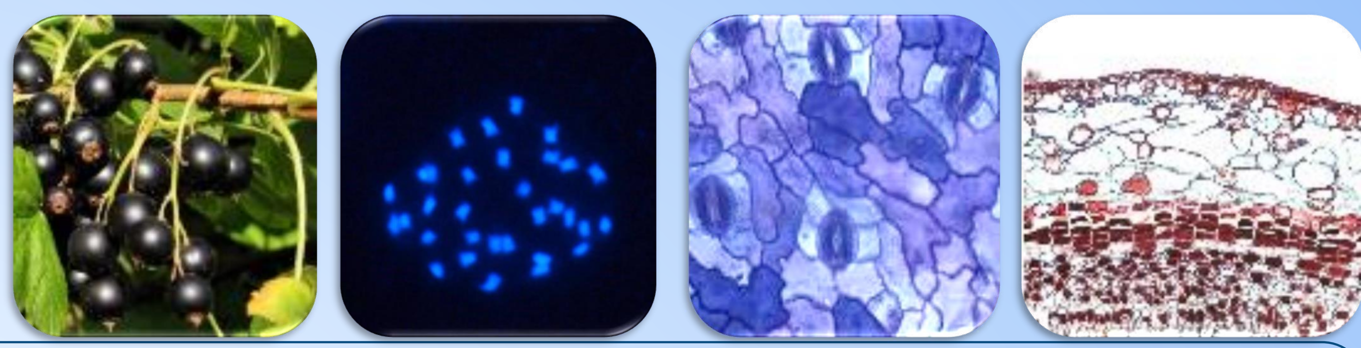
Aleksandra Machlańska, Agnieszka Marasek-Ciołakowska, Stanisław Pluta, Łukasz Seliga, Małgorzata Podwyszyńska

The National Institute of Horticultural Research, Konstytucji 3 Maja 1/3, 96-100 Skierniewice, Poland



THE EFFECT OF POLYPLOIDIZATION ON PHENOTYPIC CHARACTERISTICS OF BLACKCURRANT (*RIBES NIGRUM* L.)

INTRODUCTION

Poland is one of the world's largest producers and largest exporters of blackcurrant (*Ribes nigrum* L.). All natural species of the genus *Ribes* and cultivated commercially blackcurrant cultivars are diploid (2n = 2x = 16). Polyploidization is one of the most important sources of genetic variability in crop plants and usually has a positive effect on growth rate, size of generative and vegetative organs, and fruit yield. Homogeneous tetraploid (4x) of blackcurrant (*Ribes nigrum* L.) clones originated from two Polish cultivars: 'Gofert' and 'Polares' were obtained *via* mitotic chromosome doubling at the Research Institute of Horticultural Research (InHort) in Skierniewice, Poland. The aim of research was an assessment of neotetraploid clones of blackcurrant for selected phenotypic traits.

The ploidy level of neotetraploids derived from the Polish blackcurrant cultivars: 'Gofert' and 'Polares' was confirmed by flow cytometry. The number of chromosomes was 2n=2x=16 for diploid clones and 2n=4x=32 for tetraploid clones.

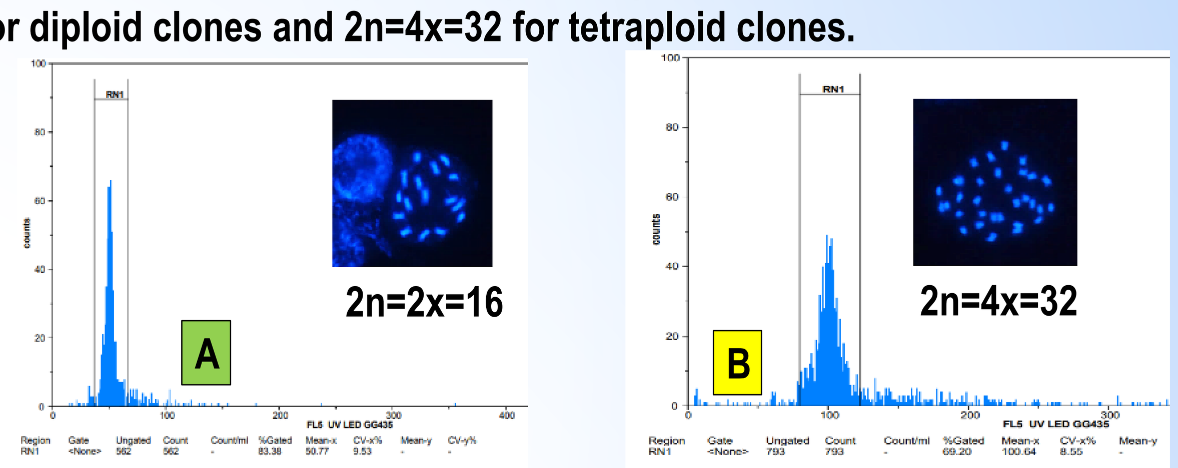


Fig. 1. Histograms of flow cytometry and metaphase chromosomes of diploid (A) and tetraploid (B) *R. nigrum* cv. 'Gofert'.

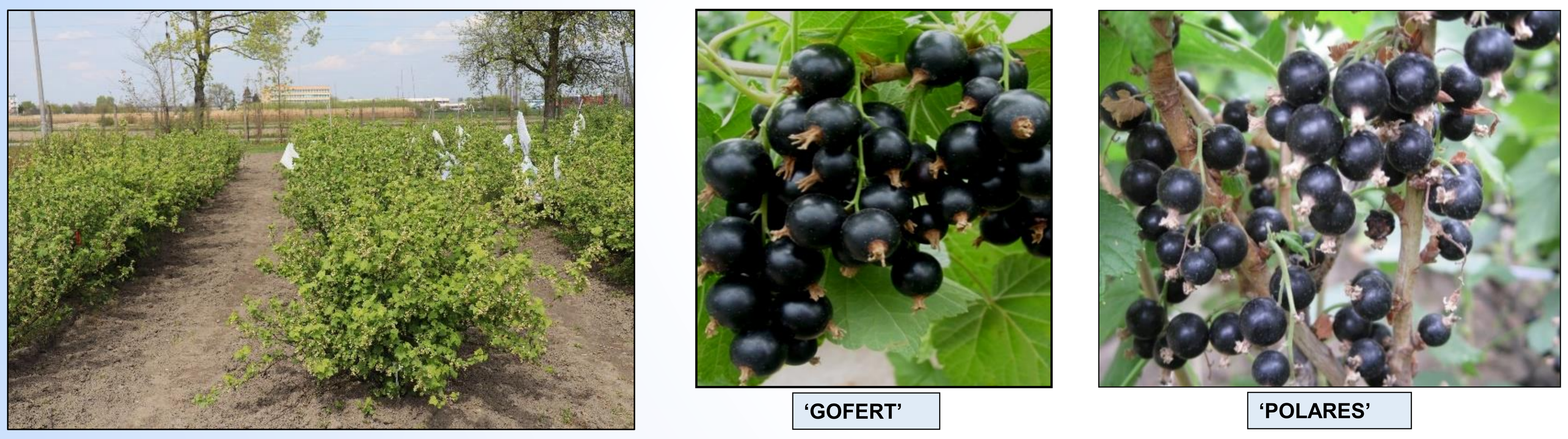


Fig. 2. Clones of 'Gofert' in field (season 6th).

MATERIALS AND METHODS

The material consisted of diploid and tetraploid blackcurrant clones derived from the Polish cultivars: 'Gofert' and 'Polares'. These cultivars are resistant to powdery mildew and white pine blister rust. Additionally, 'Polares' is genetically resistant to blackcurrant gall mite. The studies were carried out in the 5th and 6th vegetative seasons.

During the vegetative season the following traits were assessed the size of the bushes and annual shoot growth, size of leaves and the number of stomata. Observations were carried out in a light microscope. Next, the During the flowering period the following traits were assessed the time and intensity of flowering, the size and number of flowers, the size and viability of pollen, the size of fruit and the number and weight of seeds. We analyzed of pollen viability on the basis of germination of pollen grains on a medium with sucrose and after staining the cytoplasm of pollen grains with Alexander's reagent.

RESULTS

Tetraploid clones were characterized by poorer growth and vigor as compared to diploid cultivars. They bloomed slightly later and less intensively, than diploids. Leaves of induced tetraploids were smaller, had lower chlorophyll content but they were thicker than in diploids. Stomatal size was increased in tetraploid clones by 50% while stomatal density was negatively correlated with increased ploidy level. Anatomical structure of tetraploid leaf was also changed, the adaxial and abaxial epidermis, the layer of palisade and spongy mesophyll cells were thicker. Tetraploid clones were characterized by a larger diameter of pollen grains and lower viability compared to diploid initial cultivars 'Gofert' and 'Polares'. Analyzing the generative stage of plants, tetraploids had larger flowers and flower elements such as anthers, pistils compared to diploids. For tetraploids, the number of flower per inflorescence was similar but the racemes were shorted and thicker also the Berry size was similar but tetraploid clones had lower seed set ability.

Genotype	Plant height (cm)	Plant width (cm)	Annual shoot growth (cm)
Gofert 2x	125.5 a	157.0 a	16.5 a
Gofert 4x	118.1 a	151.4 a	17.0 a
Polares 2x	110.0 a	134.7 a	7.6 a
Polares 4x	65.6 b	64.4 b	9.1 b

Table 1. Characteristics of diploid (2x) and tetraploid (4x) clones of 'Gofert' and 'Polares' in vegetative stage in 2023 (season 6th).

Fig. 3. Diploid and tetraploid clones during flowering - 2021

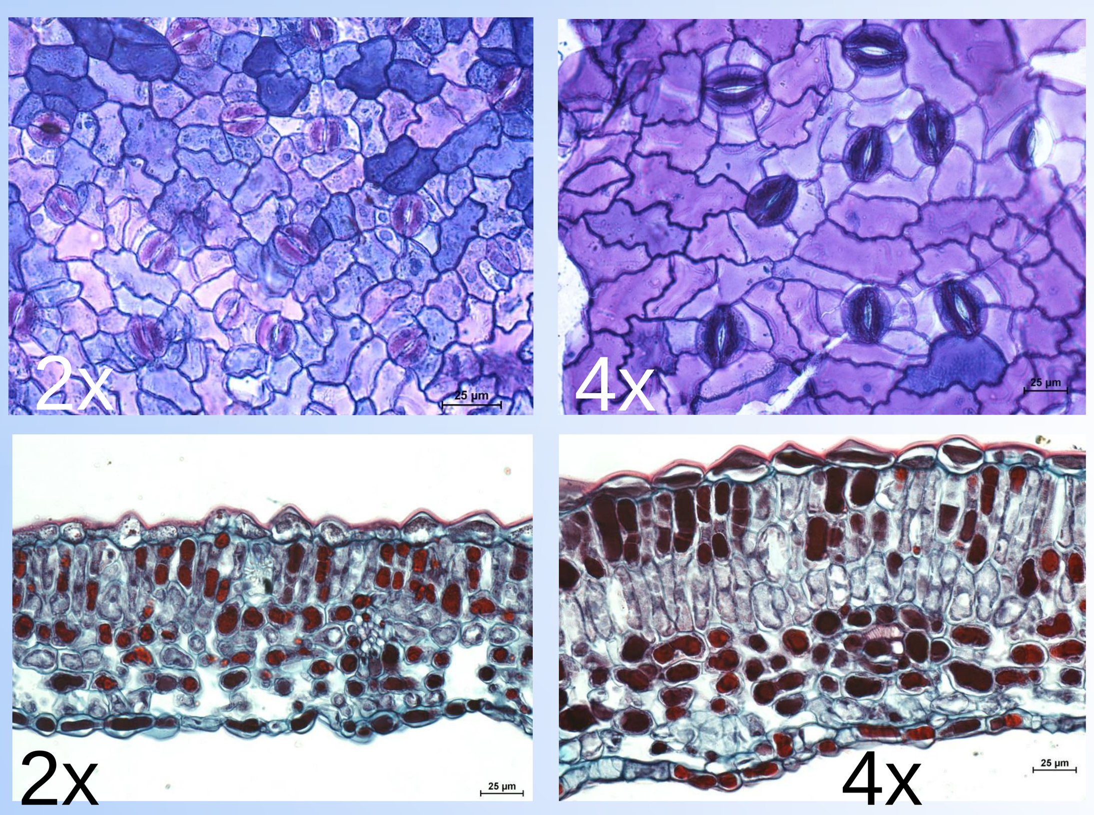


Fig. 4 Abaxial leaf surface and leaf cross sections of diploid (2x) and tetraploid (4x) cultivar 'Gofert'.

Genotype	Leaf surface (cm^2)	Stomata lenght (µm)	Chlorophyll content index (CCI)	Leaf thickness (µm)
Gofert 2x	49,8 a	20,2 a	8,7 a	121,6
Gofert 4x	55,4 a	27,8 b	12,4 b	144,2
Polares 2x	45,6 a	23,5 a	8,9 a	192,9
Polares 4x (smaller plants)	41,2 b	34,6 b	13,0 b	179,1

Table 2. Characteristics of diploid (2x) and tetraploid (4x) clones of 'Gofert' and 'Polares' in vegetative stage in 2023 (season 6th).

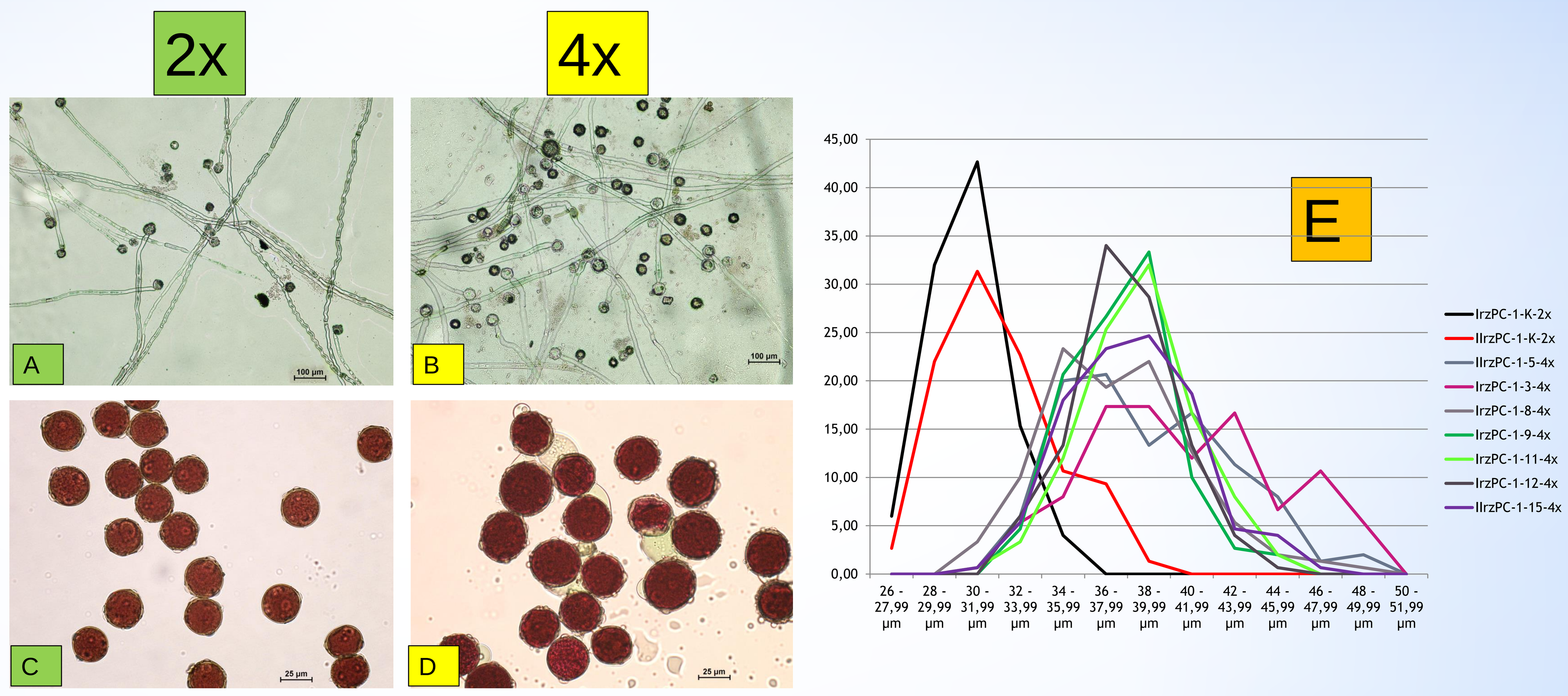


Fig. 5. Germination of pollen grains on the medium with sucrose of diploid (A) and tetraploid (B) clones of 'Gofert'. Staining the cytoplasm of pollen grains with Alexander's reagent of diploid (2x) (C) and tetraploid (4x) (D) clones of 'Gofert'. (E) pollen size distribution.

Fig. 6. Generative forms of diploid (2x) and tetraploid (4x) clones of cv. 'Polares'.

CONCLUSION AND PERSPECTIVES

In conclusion the tetraploid clones of the two examined cultivars obtained from *in vitro* polyploidization are significant step in the applied breeding program on expanding the pool of genetic variability within the *Ribes* genus.The most valuable clones will be used in the blackcurrant breeding program at the polyploidy level, potentially leading to develop of new triploids and tetraploids hybrids.

Acknowledgments

The research was carried out as part of the special-purpose subsidy of the Ministry of Agriculture and Rural Development - Task 3.7: "*Production of initial materials of blackcurrant with dessert-quality fruit. useful for trellising system cultivation and resistant to gall mite. leaf and shoot diseases*".