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

APPLICATION OF THE EMBRYO RESCUE TECHNIQUE IN BREEDING OF POLYPLOID HYBRIDS IN GENUS *RIBES*

INTRODUCTION

Blackcurrant (*Ribes nigrum* L.) is economically important fruit crop in Poland. New cultivars of black currant with increased resistance/tolerance to biotic and abiotic stresses and with improved quality of fruit, with larger, attractive fruits, better taste, delicate flesh and fewer seeds are in great demand. The sources of genetic variability in crop plants are distant crossbreeding and polyploidization. Black currant and almost all of them cultivars are diploids. Homogeneous tetraploids of cultivars 'Gofert' and 'Polares' were obtained in The National Institute of Horticultural Research in Skierniewice, Poland (Podwyszyńska and Pluta, 2019).
In the case of blackcurrant, it seems that crossbreeding between tetraploids of different cultivars is more advantageous. However, difficulties in currant breeding may arise when trying to cross tetraploid with diploid cultivars in order to obtain triploids, which may limit the possibility of breeding and creating new polyploid clones.
A common phenomenon that makes it difficult to obtain plants from distant crosses is the death of hybrid embryos in the early stages of development. Therefore, in the absence of seedlings from interploid crosses, to increase the effectiveness of crossbreeding, it also seems reasonable to attempt to use the technique of in vitro cultures of isolated embryos (embryo rescue). The aim of this study was to use embryo rescue (ER) techniques to overcome post-zygotic barriers and to prevent the embryos abortion.

MATERIALS AND METHODS

The material consisted of diploid and tetraploid blackcurrant clones derived from the Polish cultivars: 'Gofert' and 'Polares'. Crosses were made between selected clones derived from the 'Gofert' and 'Polares' cultivars involving diploid and tetraploid plants 2x × 4x, 4x × 2x and 4x × 4x. Immature embryos were isolated between 40 to 55 days after pollination. The presence of endosperm and developmental stage of embryos was estimated. Isolated embryos were transfered to solid White's medium (1943) with the addition of 20 g/L of sucharose and 1 mg/L of kinetin. Embryos were then placed on solid MS without kinetin, containing sucrose 30 g/L (Murashige and Skoog, 1962) where they were maintained up to the stage of four leaves. They were tranferred for 4 weeks on muliplication medium (MS, vitamines, inositol, BA 0.6 mg/L, succharose 30 g/L, pH 5.7) and then on rooting medium (1/2 MS, vitamines, inositol, riboflavin 5 mg/L, IBA 1 mg/l). Completely regenerated plants were acclimatized and planted ex vitro.

CONCLUSION

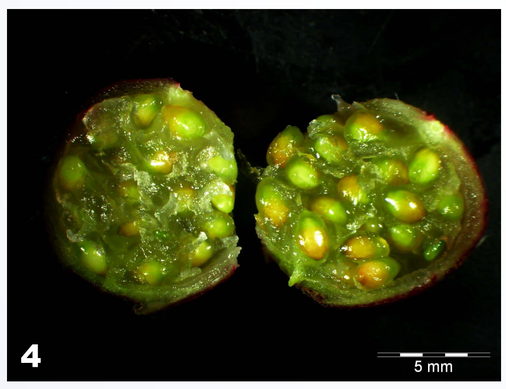
Embryo abortion was observed at the early stage of developent in the interploid crosses due to the failure of endosperm development. The highest percentage of embryos starting to grow was observed in the group of embryos isolated at the torpedo stage 55 days after pollination. Completely regenerated and adapted to in vivo conditions plants were obtained from crosses at tetraploid level (4x × 4x). Over 300 seedlings were produced and planted into the field.

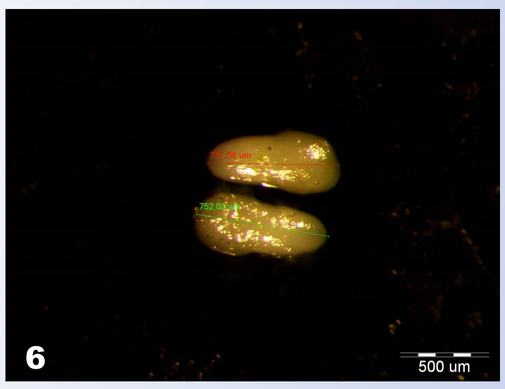
RESULTS

Fot. 1. The isolators on the black currant clones placed after the crosses carried out.

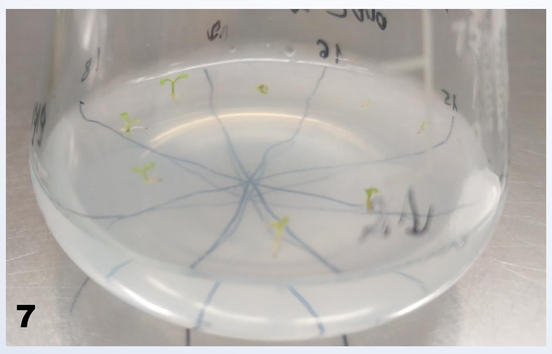
Fot. 2. Early embryo deaths in interploid crosses.

Fot. 3. Properly growing fruit from 4x × 4x crosses.

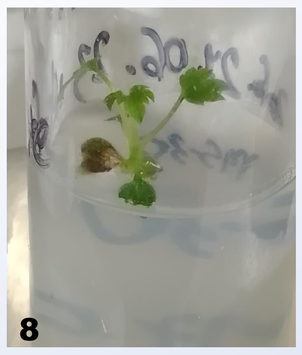




Fot. 4 – 6. Isolation of seeds and then embryos at the torpedo stage from black currant fruit.



Fot. 7. Seedling growth on White's solid medium with 20 g/L of sucrose and 1 mg/L of kinetin in a cross 'Polares' 2x × 'Gofert' 4x.



Fot. 8. Seedling growth on MS medium without hormones cross 'Gofert' 2x × 'Gofert' 4x.

Fot. 9. Seedling growth on muliplication medium (MS, vitamines, inosytol, BA 0.6mg/L, sucrose 30g/L, pH 5.7) obtained from cross 'Polares' 4x × 'Gofert' 4x.

Fot. 10-11. Seedlings growing in phytotron and acclimatized in ex vitro conditions.

Table 1. Number of seedlings generated using embryo rescue technique.

Crossbreeding	Number of seedlings
'Polares' 4x × 'Gofert' 4x	265
'Gofert' 4x × 'Polares' 4x	39
'Gofert' 2x × 'Gofert' 2x	8

References

Podwyszyńska M., Pluta S. (2019). In vitro tetraploid induction of the blackcurrant (*Ribes nigrum* L.) and preliminary phenotypic observations. Zemdirbyste-Agriculture, 106 (2): 151–158

White P.R. 1943. A Handbook of Plant Tissue Culture. J. Cattell, Lancaster. 277 p.

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