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Influence of *in vitro* propagation on the economically important traits of strawberry cv. Marmolada

Vpliv *in vitro* razmnoževanja na ekonomsko pomembne lastnosti jagod cv. Marmolada

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Abstract. *In vitro* propagation of strawberries is a promising alternative to traditional propagation, since it provides better sanitary status of plants. The possibility of somaclonal variation presents one of the drawbacks of this method, but this phenomena can be minimised by the choice of optimal tissue culture procedure.

To evaluate the influence of *in vitro* growing on economically important traits of strawberry cv. Marmolada, rooted plants were produced *in vitro* from a long term and from a newly established culture. These plants were used as mother plants for short multiplication in the field. Vegetative and generative traits of their runner plants were compared with conventionally produced cold storage plants and plants derived directly from tissue culture. Statistically significant differences were observed among different plant types in the number of runners per plant, number of flowers per plant, number of fruits per plant and yield per plant. The most pronounced differences were observed in the number of fruits per plant and in the yield per plant. Plants obtained directly from tissue culture were almost twice as productive as conventionally produced cold storage plants. Conventionally produced cold storage plants had statistically significantly higher yields and fruit number per plant than runner plants derived from micropropagated mother plants. High yields of plants obtained directly from *in vitro* were mainly the results of their significantly prolonged ripening.

Keywords: strawberry, micropropagation, yield, quality, somaclonal variation

Izvleček. *In vitro* razmnoževanje jagod predstavlja zanimivo alternativo klasičnemu razmnoževanju, saj zagotavlja boljše zdravstveno stanje sadik. Somaklonska variabilnost je lahko omejujoč dejavnik pri uveljavljanju mikrorazmnoževanja jagod, vendar lahko z izbiro optimalne *in vitro* tehnike ta pojav znižamo na minimum.

Za ugotavljanje vpliva *in vitro* gojenja na ekonomsko pomembne lastnosti jagod cv. Marmolada smo vzgojili ukoreninjene rastlinice iz dolgotrajne in novo inicirane kulture te sorte in jih posadili v matični nasad. Vegetativne in generativne lastnosti njihovih hčerinskih rastlin smo primerjali z vegetativnimi in generativnimi lastnostmi standardno vzgojenih hlajenih sadik in rastlin, posajenih v poskus neposredno iz tkivne kulture. Med različnimi tipi sadik smo ugotovili statistično značilne razlike v številu živic na rastlino, številu cvetov na grm, številu plodov na grm in pridelku na grm. Največje razlike smo opazili v številu plodov na grm in pridelku na grm. Rastline, izvirajoče neposredno iz tkivne kulture, so imele skoraj enkrat večji pridelek v primerjavi s standardno vzgojenimi hlajenimi sadikami. Te so v številu plodov na grm in v pridelku na grm statistično značilno presegle hčerinske rastline matičnih rastlin, vzgojenih *in vitro*. Visoki pridelki rastlin, posajenih neposredno iz *in vitro* pogojev, so bili predvsem posledica njihovega močno podaljšanega zorenja.

Ključne besede: jagode, mikrorazmnoževanje, pridelek, kakovost, somaklonska variabilnost

Introduction

Strawberries can only be multiplied by vegetative methods, which explains the great dissemination of parasites. Most of strawberry producing countries thus have drawn up a program of certification to provide healthy planting material, which requires a minimum delay of 3 years before a healthy cultivar is at producer's disposal (Boxus & al. 1977). *In vitro* propagation of strawberries can speed up the propagation process. The possibility of somaclonal variation presents one of the drawbacks of this method. Mainly epigenetic changes such as hyperflowering, smaller fruit size and lower yields have been reported to occur. The quality of the material obtained *in vitro* and its behaviour in the field depends on genotype and tissue culture procedure (López-Aranda & al. 1994).

To determine the influence of tissue culture on field performance of cv. Marmolada, traditionally produced cold storage plants were compared with plants either derived directly from tissue culture or from micropropagated plants further propagated for one cycle in the nursery.

Materials and methods

Plant propagation

Meristems from terminal buds of dormant plants of cv. Marmolada, one of the main varieties cultivated in Slovenia, were isolated in December 1999 and grown *in vitro*. In the beginning of February 2000, newly developed shoots were multiplied according to the procedure described by Boxus & al. (1977). The same was done with shoots of cv. Marmolada grown *in vitro* over 3 years. Multiplied shoots originating from both newly established and old culture were transferred to a rooting medium for strawberries (Boxus & al. 1977). On 19th of April 2000 rooted shoots were transferred to soil. After 14 days of acclimatisation plants were transferred in larger pots and maintained in nethouse until beginning of August, when they were planted in the nursery. In spring 2001 runner plants were separated from mother plants and stored at -1° C until planting in the experimental field.

On 20th of June 2000 a new *in vitro* culture of cv. Marmolada was established from meristems excised from runner tips of field grown plants. Shoots were multiplied and rooted according to Boxus & al. (1977). On 1st of March 2001 rooted shoots were transferred to soil. After 14 days of

acclimatisation plants were transferred to larger pots and grown in the greenhouse until planting in the experimental field.

Field trial

On 22nd of May 2001 the field trial was planted on the Experimental Orchard of Agricultural Institute of Slovenia at Brdo near Lukovica. Four different plant types of strawberry cv. Marmolada were tested:

A. micropropagated plants originating from the *in vitro* culture established in June 2000,
B. runner plants from micropropagated mother plants, originating from the *in vitro* culture established in December 1999,
C. runner plants from micropropagated mother plants, originating from a culture maintained *in vitro* over 3 years,
D. conventionally produced cold storage plants of A quality.

A randomised block design with 4 treatments (plant types), 4 replications and 10 plants per plot was used. Following data were recorded: number of runners per plant, number of flowers per plant, number of fruits per plant and yield per plot. The STATGRAFICS Plus version 3.1 computer programme was used for statistical analyses. The analysis of variance was performed for the number of runners, number of flowers on 26th of July 2001, number of fruits and yield.

Results and discussion

Sanitary status of all plants included in the trial was very good. As expected, mutations were not observed, since propagation *in vitro* through axillary branching is generally considered to produce genetically stable material. Nevertheless, yellow leaf variants were reported to occur among micropropagated plants of strawberry cv. Redcoat by Nehra & al. (1994).

Considerable differences in vigour were observed among different plant types. Plants derived directly from tissue culture (plant type A) showed uniform and extremely vigorous growth with large leaves and very large flowers. Control plants (plant type D) were also uniform in vigour and had large leaves and flowers. In contrast, runner plants derived from micropropagated mother plants (plant type B and C) exhibited weak and variable growth and had small leaves and very small flowers.

Runner plants derived from micropropagated plants also produced the lowest number of flowers per plant as recorded on July 26th 2001 (Tab. 1). Plants of the type C had statistically significantly lower number of flowers per plant in comparison with plants derived directly from tissue culture as well as with traditionally propagated plants (Tab. 1). The number of flowers per plant of plant type B differed statistically significant only from plants obtained directly from tissue culture (Tab. 1). Since in strawberry not all of the flowers develop fruits, the number of fruits per plant was lower than the number of flowers recorded for all the plant types with exception of plants derived directly from tissue culture. The latter exhibited prolonged flower development. Consequently fruit ripening was also markedly prolonged. Fruits of plants derived directly from tissue culture were harvested from 4th of July until 24th of August whereas fruit ripening of other plant types ended more or less by 2nd of August. Plants derived directly from tissue culture gave statistically significant higher number of fruits and higher yields per plant than all other plant types including conventionally produced

cold storage plants mainly due to prolonged ripening (Tab. 1). In spite of long ripening time of type A plants there was very little variation in fruit number and fruit yield among picking dates. Fruit weight diminished only slightly towards the end of the ripening time.

Table 1: Vegetative and generative traits of different plant types of strawberry cv. Marmolada in the field

Plant type	Number of runners per plant	Number of flowers per plant	Number of fruits per plant	Yield per plant in g
A	10.1 a	13.4 a	16.15 a	97.6 a
B	7.0 c	8.1 bc	6.45 c	31.1 c
C	9.3 ab	7.3 c	6.25 c	30.9 c
D	7.6 bc	10.5 ab	9.0 b	54.2 b

Mean separation within column by Duncan's multiple comparison procedure
Values followed by a same letter do not differ significantly, $P < 0.05$

In the experiment of KARHU & HAKALA (2002) micropropagated plants of cv. Senga Sengana, planted directly in the field, also showed more abundant flowering and gave statistically significant higher marketable crop than control runner plants in the first cropping year. However no differences in yielding were recorded between micropropagated and control runner plants in cv. Zefyr (KARHU & HAKALA 2002). Similarly, an increased productivity of directly used micropropagated plants that was not accompanied by reduced weight was observed by NEHRA & al. (1994) for cv. Redcoat, whereas the yielding of cv. Veestar was not affected by the propagation method. In contrast to the results of KARHU & HAKALA (2002), SZCZYGIEŁ & al. (2002) recorded higher yields but diminished fruit quality of micropropagated plants of cvs. Senga Sengana, Kent and Dukat in comparison with the traditionally propagated runner plants. LÓPEZ-ARANDA & al. (1994) and SZCZYGIEŁ & al. (2002) quote other authors who observed a sharp decrease in average fruit weight, which was usually limited mainly to the plantlets coming directly from micropropagation. SZCZYGIEŁ & al. (2002) therefore suggested, that micropropagated plants should at least once be reproduced by runners before planting in the field. In our experiment runner plants derived from mother plants originating from either old or newly established *in vitro* culture both behaved poorly. The low vigour, flowering and yield of these plants were probably the result of the late planting of mother plants in the nursery and/or unfavourable weather condition during winter. As a consequence runner plants could not fully develop. Earlier planting of micropropagated plants in the nursery was prevented by poor development of rooted plantlets after acclimatisation due to extremely high temperatures.

Conclusions

Plants of cv. Marmolada derived directly from tissue culture showed markedly prolonged flower and fruit development. The number of fruits and yield of these plants were therefore almost twice as high as in conventionally produced cold storage plants, which showed statistically significantly better productivity than runner plants derived from micropropagated mother plants. The low vegetative and generative characteristics of the latter were probably the result of the late planting of mother plants in the nursery and/or unfavourable weather condition during winter.

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