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PRIMJENA TEHNIKE MIKRORAZMNOŽAVANJA U UZGOJU SEZONSKOG I TRAJNOG CVIJEĆA S PRIMJERIMA RADA LABORATORIJA ZA TKIVNE KULTURE "SBW INTERNATIONAL BV"

APPLICATION OF MICROPROPAGATION TECHNIQUE IN BREEDING OF SEASONAL AND EVERGREEN FLOWERS WITH EXAMPLES FROM SBW INTERNATIONAL BV TISSUE CULTURE LABORATORY

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SAŽETAK

Komercijalna hortikulturna proizvodnja razvila se do stupnja kada proizvedene kulture moraju biti ujednačene, dobre kakvoće i visoke prodajne vrijednosti. Ali nerijetko mali izdanci cvijetnih kultura umnoženi konvencionalnim metodama osjetljivi su na bolesti kada se nađu u smjesi za ukorjenjivanje, što rezultira s velikim gubicima. Još više, potrebno je nekoliko godina za dobivanje adekvatnih matičnjaka iz izdanaka novodobivenih kultura. Naprotiv, veliki broj hortikulturnih biljaka proizvedenih metodom kulture tkiva kao jedinim industrijskim procesom za proizvodnju biljaka, uspješno ispunjavaju tržišne standarde. Tako je mikrorazmnožavanje postalo važan dio komercijalnog razmnožavanja velikog broja biljaka. Prednosti mikrorazmnožavanja kao sistema za umnožavanje mogle bi se sumirati u sljedećem: visoko brojčano umnožavanje specifičnih klonova; proizvodnja zdravih biljaka; umnožavanje matičnjaka za proizvodnju hibridnog sjemena, osiguravanje izvansezonske (cjelogodišnje) proizvodnje rasadnog materijala, dugotrajno čuvanje zdravog biljnog materijala.

Visoko mjesto ima in vitro laboratoriji, koji pokušavaju riješiti neke proizvodne probleme uzgajivača cvijeća, SBW International BV čiji proizvodni dio je SBW Vinica Vitro. SBW International BV uspješno razmnožava

preko 350 vrsta cvijeća, veliki dio su trajnice, prikladne za kultiviranje urbanih prostora. Neke od njih su: Agapnthus, Brunerra, Dahlia, Curcuma, Delphinium, Eringium, Geranium, Hemorocallis, Polemonium, Saxifraga, Persicaria, Globa, Tacca, Cotinus, Ilex, i dr.

U ovome radu glavni razlozi primjene ove tehnike bit će objašnjeni kroz nekoliko cvjetnih vidova (Aster, Dahlia, Petunia, Eringium, Brunerra, Geranium, Astrantia), brojčanom povećanju plantleta tih vidova kroz nekoliko ciklusa mikropropagacije do faze ukorjenjivanja.

Ključne riječi: tkivne kulture, in vitro, mikropropagacija, perene, bezvirusne biljke, propagacijski faktor

ABSTRACT

Commercial horticulture has progressed to the stage where crops must be uniform, of good quality and give high saleable yields. But not seldom cuttings of horticultural crops propagated by conventional methods are susceptible to disease in the rooting mixture resulting in many losses. Moreover, several years are required to produce adequate stock plants using cuttings of newly released cultivars. Contrary, many horticultural plants produced in tissue culture, as the only industrial plant production process successfully fulfill market standards. According to that, micropropagation has become an important part of commercial propagation for many plants. The advantage of micropropagation as a propagation system can be summarized as: mass propagation of specific clones; production of pathogen free plants; clone propagation of parental stock for hybrid seed production; provide year-round nursery production; long term storage of disease free stock. High position among tissue culture laboratories that try to solve some production problems of horticultural breeders is held by "SBW International BV" whose production part is "SBW Vinica Vitro".

SBW successfully propagates over 350 species and many of them are perennial, suitable for cultivation of urban areas. Some of them are: Agapanthus, Brunnera, Dahlia, Curcuma, Delphinium, Eringium, Geranium, Hemmerocallis, Polemonium, Saxifraga, Persicaria, Globa, Tacca, Cotinus, Ilex, and many others.

In this article main reasons for tissue culture will be explained on several horticultural species (Aster, Dahlia, Petunia, Fryngium, Brunerra, Geranium, Astrantia) as well as the way the number of plantlets of those species increases through several cycles of propagation to the rooting stage.

Key words: tissue culture; in vitro; micropropagation; perennial crops; bedding plants; virus free plants; propagation factor, annual crops.

INTRODUCTION

Tissue culture is an aseptic laboratory procedure that requires unique facilities and special skills. There are number of reasons to produce plants in tissue culture. These include:

- Micropropagation;
- Somatic embryogenesis;
- Natural product synthesis for such chemicals as natural flavors, colors, pharmaceuticals (medicines) and even plastics;
- Embryo rescue;
- Anther (microspore) culture to produce haploid plants for crop breeding;
- Micro grafting; and
- Crop improvement through biotechnology. This includes gene transfer, somaclonal variation and protoplast fusion.

Of these techniques, the two most directly related to plant propagation are micropropagation and somatic embryogenesis.

The following description is still frequently used to describe micropropagation: "Small pieces of plant are removed from a selected parent and sterilized to remove contaminants and diseases. Then, they are placed in sterile culture vessels containing a gel based medium that contains all things necessary for ideal growth (including carbohydrates, organic and inorganic nutrition and growth regulators). The cultures are placed in growth room with controlled temperature, irradiance and day length so that in its ideal culture, the plant is capable of unrestricted (exponential) growth".

Over recent years, micropropagation has passed through various stages in its development.

Fig. 1. Dahlia sp.- *multiplication stage*
Sl.1. Dahlia sp.- *multiplikacijska faza.*

Fig.2. Petunia sp.- *multiplication stage*
Sl.2. Petunia sp.- *multiplikacijska faza*

Fig.3. Eryngium sp.- *multiplication stage*
Sl.3. Eryngium sp.- *multiplikacijska faza*

Fig.4. Brunnera sp-. *multiplication stage*
Sl.4. . Brunnera sp-. *multiplikacijska faza*

CHARACTERISTICS OF MICROPROPAGATION

Micropropagation has become an important part of commercial propagation for many plants and may be used for:

1. Mass propagation of specific clones,
2. Production of pathogen-free plants,
3. Clonal propagation of parental stocks for hybrid seed production,
4. Year round nursery production,
5. Germplasm preservation.

1. Mass propagation of specific clones.

The objective of commercial propagation is to reproduce copies of an original parent plant. The controlled aspects of micropropagation permit the rapid propagation of individuals from a single plant. Multiplication rates can be very high, since plants in culture can theoretically be multiplied at an exponential rate by consecutive sub culturing (e.g. one month apart). Although such theoretical rates are not usually maintained in practice, the actual rates that can be attained under proper management are impressive. Commercial micropropagation is particularly useful for the following:

a. Plants whose natural rate of increase is relatively slow: orchids; bulbs; gerbera; many foliage plants; herbaceous; perennials; palm species. For example the major drawback in the orchid industry has been the length of time taken for clonal propagation of selected species and hybrids. It takes about 10 years to cultivate about a dozen good-sized propagations. A good example can be seen in the herbaceous perennial-*Hosta*. Traditionally, this plant is propagated by division. Division yield is fewer than ten plants from the original mother plant per year. Micropropagated *Hosta* can yield a similar multiplication rate every month.

b. New cultivars where commercial demands require getting to market in as short time as possible. This has been important for number of crops. Vegetative cloning avoids the possibility of rearrangement of genetic material consequent upon sexual reproduction, so that in vitro micropropagation techniques do offer the opportunity of rapid cloning of selected genotypes. Application of such methods would increase breeding and selection efforts.

c. Cultivars whose high value makes micropropagation a viable alternative to conventional methods. Micropropagation is relatively expensive compared to other methods of propagation. But micropropagation may be the method of choice for reasons other than cost. Examples include especially woody plants such as valuable rootstocks, rhododendron, and special perennials such as day lily.

d. Propagation of difficult-to-root plants.

Many woody plants fail to root from cuttings. Traditionally grafting has been the only method to clonally propagate these plants. Micropropagation offers a viable alternative for propagation of these plants on their own roots.

e. Conservation of endangered species.

Micropropagation can provide a rapid method for the multiplication of endangered or threatened species. In these cases only a few plants may be available as stock plants. Micropropagation can also be an alternative to collecting plants from natural habitats to supply commercial markets.

2. Production of pathogen-free plants

Production of propagation material free of fungal, bacterial and systemic virus infections has become an essential aspect of propagation.

Micropropagation provides a method to rid a clone of external pathogens and can also provide a system in which plants are kept free of reinfection until delivery. Without using methods against pathogens, for example, virus infection accumulated from year to year can result in the degeneration of the species, decreasing yield and deterioration in reproductive ability. Important ornamental cultures (carnation, chrysanthemum, orchid, tulip and other plants) are very often completely infected with viruses and are subject to a complex infection. The two-phase method of plant regeneration from meristem (1. isolation and cultivation apical meristem, and 2. root induction on regenerated shoots) ensures the production of virus-free plants without the additional stage of thermotherapy (Rybalko, 1992).

Propagators should not automatically assume systemic pathogens are absent unless culture index for bacteria, fungi or viruses are made.

Fig. 5. Geranium sp.- *multiplication stage.*
Sl.5. Geranium sp.- *multiplikacijska faza.*

Fig.6. Astrantia sp.-*multiplication stage*
Sl.6. . Astrantia sp.- *multiplikacijska faza*

3. Clonal propagation of parental stocks for hybrid seed production.

4. Provide year-round nursery production.

Most nursery operations are seasonal. Micropropagation has the potential for continuous year-round operation with production scheduled according to market demands. High-volume production requires high-volume distribution and the facilities to stock-made items. Combining production with cold storage facilities makes it possible to hold material for peak marketing periods.

Propagation by vegetative means is an important technique in roses. Taking of cuttings and budding outdoors are confined to the warmer periods of the year. In mild climates cuttings can be taken throughout the summer and autumn or from plants grown in glasshouse throughout the year. Hardwood cuttings are made from the previous season's shoots, softwood cuttings from the current seasonal growth. Such dependence upon climatical conditions is overcome by micropropagation.

5. Germplasm preservation

The major method of germplasm preservation is preservation of seeds. However, seed storage requires a relatively large space with special storage conditions. Also, seed storage is not an option for recalcilant seeds. For these reasons, preservation of vegetative tissue as explants is an attractive alternative. Cryopreservation, often using antifreeze materials as an aid, has been used to ultra freeze vegetative tissue in a fashion similar to that of seeds (Kester et al., 1997).

ECONOMICS OF MICROPROPAGATION FOR THE HORTICULTURAL INDUSTRY

Micropropagated plants cannot compete in price with plants originating from seed, but may do so against more complexly produced vegetative material. Actually, if there are additional benefits from the use of

micropropagated materials, then this provides areas for successful competition with products of macro-vegetative propagation. Even more, micropropagation can improve macro methods where these produce complete rooting, and are reliably devoid of seasonal effects. Micropropagation is a method that provides plants of difficult or unusual types, with advantages obtained from high health status. Macropropagation compared with micropropagation is more straightforward, involving fewer stages, but only one plant is achieved per cutting. Micropropagation is far more flexible, permitting recycling and building up large stocks very quickly.

Advantages of micropropagation compared with macropropagation may be summarized as:

1. Rapid multiplication rate,
2. Flexible process,
3. Large number of plants produced quickly,
4. High health status,
5. Effective with difficult subjects.

Disadvantages of micropropagation are:

1. Delicate product, possessing a thin cuticule and few root hairs,
2. Skillful staff and high grade facilities,
3. Complex mother stock care to prevent pest pathogen contamination,
4. Prolonged establishment period after in vitro phase.

Four categories of micropropagation can be identified in relation to the economics of production:

1. Micropropagation which is economically superior to conventional methods, e.g. *Nephrolepsys* or *Kalanchoe spp*;

2. Micropropagation which is economically inferior to macropropagation e.g. *Saintpaulia ionantha*;

3. Micropropagation where there is added value other than benefits of easier propagation e.g. improved sanitation, as in the production of virus-tested material of *Begonia elator* hybrids, *Pelargonium* hybrids, *Narcissus* cultivars;

4. Micropropagation used as a tool for programs with wider objectives e.g. cloning mother plants, which are normally produced from seeds, thereby enabling desirable individuals to be identified and multiplied rapidly, to provide reliable agronomic merits such as increased growth rate or pest and

pathogen resistance. Economic limitations to micropropagation may be summarized on the basis of disadvantage of this method, and they are:

1. Necessity for skilled personnel, using intensive methods leading to high labor cost;
2. Required high initial investment costs in laboratory and quality buildings and equipment, associated with glasshouse facilities, equipped to handle large numbers of delicate propagules rapidly;
3. A propagation system which can be very sensitive to infections in vitro from micro-organisms, present externally and internally on plant material and at transfer ex vitro, which can lead to extensive losses when large number of plants are cultivated in a small area;
4. Regeneration requirements which differ between cultivars and varieties of the same species on a defined medium, leading to high development costs for the introduction of expanded systems for new subjects;
5. Prolonged cultivation and careful husbandry required in the weaning and acclimatization phases.

PLANT MICROPROPAGATION AT SBW INTERNATIONAL BV

SBW International BV is an organization that offers many types of services to its principals. Its services include: propagation, disease eradication, germplasm conservation, in vitro fertilization, in vitro germination of seed, all kinds of embryo-rescue techniques and production of haploid, polyploid or mutated plants. The major activity is plant micropropagation. The company produces many ornamental, agricultural, tropical and vegetable plants for its principals, mainly located in The Netherlands, but some are spread all over the world. Every year ca 1500 new varieties are brought into tissue culture. SBW clients are breeders, selectors and propagators in horticultural plants. For new crops a complete protocol for micropropagation is developed or an existing one is adapted. Some varieties are cleaned from pathogens such as viruses, fungi or bacteria. Other varieties, especially belonging to crops like Petunia and Verbena are certified according to the NAKB-Elite® system. Whatever type of initiation is used from every clone obtained in vitro, a test sample of plants is sent to the client who checks their identity and trueness to type. This procedure

ensures that every clone from each successfully established initiation of a variety is checked before large-scale production is started. SBW has the capacity and know-how for large-scale micropropagation. The product itself may be rooted plants in a tissue culture vials or weaned plants from the greenhouse. SBW is ISO-9001 certified. This ensures its services follow procedures in agreement with international recognized standards for production and research. The products themelves can also be certified in close co-operation with the Dutch Inspection Service for Floriculture and Arbiculture (NAKB) SBW is able to produce NAKB-Elite®-certified plants. These plants have been tested in a way prescribed and supervised by the NAKB for all known pathogens to the crop and were found to flower homogeneosly and true to type. Plants provided with the certificate can be shipped all over the world.

Many different crops suitable for cultivation of urban areas are produced on a large scale at SBW International. Some of them are: *Agapanthus, Brunnera, Dahlia, Curcuma, Delphinium, Eryngium, Geranium, Hemerocallis, Polemonium, Saxifraga, Persicaria, Globa, Tacca, Cotinus, Ilex, Aster, Astrantia, Petunia*, and many others.

a. Presentation of micropropagation of annual plants: *Dahlia* and *Petunia*.

Most annual plants are propagated in the laboratory to provide healthy, disease free plants. SBW produce plants which are sold to young plant companies that use their plants as so called mother stock plants. From the mother stock plants cuttings are taken and sold rooted or unrooted to commercial growers.

Initial explants for multiplication of annual plants when disease eradication is required are top cuttings (apical meristem). In other cases of micropropagation, usually part of the stem consisting of a node and at least two buds are initiated to tissue culture.

Petunia varieties that are initiated for micropropagation at SBW International BV are certified according to the NAKB-Elite® system.

Generally, cultivars of this crop are divided into two groups. *Grandiflora* petunias have very large flowers, generally to 10cm across. Many are susceptible to rain damage and are best grown in sheltered hanging baskets and containers. *Multiflora* petunias are bushier than *Grandiflora* petunias, with smaller flowers to 5 cm across. They are usually more tolerant to wet weather

and are ideal for summer bedding or in a mixed border. Individual plants may carpet an area up to 1m across.

How multiplication of *Petunia* looks like on the base of its propagation factor but on contamination factor too, is visible from table 1.

Table 1. Increasing plantlets number of *Petunia* till reaching ordered amount.
Tablica1. Povećanje broja plantleta *Petunia-e* do postizanja naručene količine.

CA-pf	4	CA-pf - propagation factor in multiplication phase CA-pf - propagacijski faktor u fazi multiplikacije GA-pf - propagation factor for rooting phase GA-pf - propagacijski faktor u fazi zakorjenjivanja CA-cont. - contamination in multiplication phase CA- cont. - kontaminacija u fazi multiplikacije CD-cont. - contamination in phase before rooting CD-cont. - kontaminacija u fazi prije zakorjenjivanja GA-cont. - contamination in rooting phase GA- cont. - kontaminacija u fazi zakorjenjivanja	
GA-pf	2		
CA-cont.	2%		
CD-cont.	2%		
GA-cont.	2%		
Starting material Početni materijal	24		
Required amount Tražena količina	5400		
CYCLE	WEEK	AMOUNT	ACTIVITY
I	15 2003	24	ca
II	19 2003	92	ca
III	23 2003	362	ca
IV	27 2003	1420	ca
V	31 2003	1391	ca/cd
VI	35 2003	5510	ga
VII	39 2003	5400	la

Dahlias are grown for their flower heads, cultivated in a variety of forms and in colors from white to red, orange to yellow and pink to dark purple. They flower from mid-summer to autumn (until the frost in cool-temperate regions), when many other plants are past their best. Although often informally divided into two types: tall growing "border dahlias and low-growing "bedding" dahlias- are all good for garden display and cutting.

Bedding dahlias may be raised from seed and treated as annuals that flower from early or mid-summer to autumn. They are suitable for mass plantings for edging borders or for growing in containers. Propagation factor in multiplication phase for Dahlias is 2-8 and for rooting phase from 2-5.

Reaching the ordered amount of plantlets for the client is shown in table 2.

Table 2. Increasing plantlets number of *Dahlia sp.* till reaching ordered amount.
Tablica 2. Povećanje broja plantleta *Dahlia-e* do postizanja naručene količine.

CA-pf	2	CA-pf - propagation factor in multiplication phase CA-pf - propagacijski faktor u fazi multiplikacije GA-pf - propagation factor for rooting phase GA-pf - propagacijski faktor u fazi zakorjenjivanja CA-cont. - contamination in multiplication phase CA- cont. - kontaminacija u fazi multiplikacije CD-cont. - contamination in phase before rooting CD-cont. - kontaminacija u fazi prije zakorjenjivanja GA-cont. - contamination in rooting phase GA- cont. - kontaminacija u fazi zakorjenjivanja	
GA-pf	8		
CA-cont.	8		
CD-cont.	8		
GA-cont.	5		
Starting material Početni materijal	20		
Required amount Tražena količina	8620		
CYCLE	WEEK	AMOUNT	ACTIVITY
I	29 2002	17	ca
II	33 2002	32	ca
III	37 2002	59	ca
IV	41 2002	109	ca
V	45 2002	200	ca
VI	49 2002	368	ca
VII	01 2003	677	ca
VIII	05 2003	1245	ca
IX	09 2003	1146	ca/cd
X	13 2003	9074	ga
XI	17 2003	8620	la

b. Micropropagation of Perrenials – *Eryngium, Brunnera, Geranium* and *Astrantia* Perennial plants are usually produced through tissue culture for:

- Producing large quantities in a relatively short period (market introduction),
- Propagation varieties which do not have a high propagation factor under natural conditions,
- Increase vivo growth and vegetative and generative development.

Eryngium-is one of perennial crops that are propagated in SBW International BV for several clients. *Eryngiums* are striking plants for naturalization; some also provide long-lasting displays for a border.

Table 3 presents micropropagation of *Eryngium* through a few cycles.

Table3. Increasing plantlets number of *Eryngium* till reaching ordered amount.
Tablica 3. Povećanje broja plantleta Eryngium-a do postizanja naručene količine.

CA-pf	2	CA-pf - propagation factor in multiplication phase CA-pf - propagacijski faktor u fazi multiplikacije GA-pf - propagation factor for rooting phase GA-pf - propagacijski faktor u fazi zakorjenjivanja CA-cont. - contamination in multiplication phase CA- cont. - kontaminacija u fazi multiplikacije CD-cont. - contamination in phase before rooting CD-cont. - kontaminacija u fazi prije zakorjenjivanja GA-cont. - contamination in rooting phase GA- cont. - kontaminacija u fazi zakorjenjivanja	
GA-pf	2		
CA-cont.	3%		
CD-cont.	3%		
GA-cont.	3%		
Starting material Početni materijal	80		
Required amount Tražena količina	206850		
CYCLE	WEEK	AMOUNT	ACTIVITY
I	07 2002	76	ca
II	11 2002	147	ca
III	15 2002	285	ca
IV	19 2002	553	ca
V	23 2002	1074	ca
VI	27 2002	2083	ca
VII	31 2002	4041	ca
VIII	35 2002	7839	ca
IX	39 2002	15207	ca
X	43 2002	29501	ca
XI	47 2002	57233	ca
XII	51 2002	111032	ca
XIII	03 2003	107701	ca/cd
XIV	07 2003	213247	ga
XV	11 2003	206850	la

Brunnera – is a genus of about 3 species of rhizomatous perennials from woodland in E. Europe and N. W. Asia, valued for their flowers and ground-covering foliage. They usually have oval, rough-hairy basal leaves and lance-shaped to oval stem leaves. Terminal, cyme-like panicles of purple-blue, rarely white flowers are born in mid and late spring. Grow in woodland or a border Brunnera is fully hardy crop.

Table 4 presents increase of *Brunnera* plantlets number in the period of 32 weeks.

Table 4. Increasing plantlets number of *Brunnera* till reaching ordered amount
Tablica 4. Povećanje broja plantleta *Brunnera-e* do postizanja naručene količine

CA-pf	2	CA-pf - propagation factor in multiplication phase CA-pf - propagacijski faktor u fazi multiplikacije GA-pf - propagation factor for rooting phase GA-pf - propagacijski faktor u fazi zakorjenjivanja CA-cont. - contamination in multiplication phase CA- cont. - kontaminacija u fazi multiplikacije CD-cont. - contamination in phase before rooting CD-cont. - kontaminacija u fazi prije zakorjenjivanja GA-cont. - contamination in rooting phase GA- cont. - kontaminacija u fazi zakorjenjivanja	
GA-pf	1.9		
CA-cont.	2%		
CD-cont.	2%		
GA-cont.	2%		
Starting material Početni materijal	67		
Required amount Tražena količina	101300		
CYCLE	WEEK	AMOUNT	ACTIVITY
I	16 2002	67	ca
II	20 2002	131	ca
III	24 2002	257	ca
IV	28 2002	505	ca
V	32 2002	989	ca
VI	36 2002	1939	ca
VII	40 2002	3800	ca
VIII	44 2002	7447	ca
IX	48 2002	14597	ca
X	52 2002	28610	ca
XI	04 2003	56075	ca
XII	08 2003	54953	ca/cd
XIII	12 2003	103367	ga
XIV	11 2003	101300	la

Geranium- is a genus of about 300 species and except for annuals and biennials some of its species are herbaceous, semi evergreen and evergreen, sometimes tuberous perennials. *Geranium* flowers are white, pink, purple or blue, usually saucer-shaped, sometimes flat or star shaped, with petals sometimes reflected and often contrastingly veined or marked. Geraniums are

generally long-lived versatile and undemanding plants. Compact perennials, to about 15cm tall are good for a rock garden. Taller, clump-forming species and hybrids are suitable for a border or among shrubs.

Pererial-*Geranium* plantlets are produced in SBW International BV and their number rapidly increases on the basis of propagation factor 2.5 for the period of 4 weeks (one cycle). For the period of 11 cycles the number of young plantlets increases till 125000.

Table 5. Increasing plantlets number of *Geranium* till reaching ordered amount.
Tablica 5. Povećanje broja plantleta *Geranium-a* do postizanja naručene količine.

CA-pf	2,5	CA-pf - propagation factor in multiplication phase CA-pf - propagacijski faktor u fazi multiplikacije GA-pf - propagation factor for rooting phase GA-pf - propagacijski faktor u fazi zakorjenjivanja CA-cont. - contamination in multiplication phase CA- cont. - kontaminacija u fazi multiplikacije CD-cont. - contamination in phase before rooting CD-cont. - kontaminacija u fazi prije zakorjenjivanja GA-cont. - contamination in rooting phase GA- cont. - kontaminacija u fazi zakorjenjivanja	
GA-pf	2,5		
CA-cont.	2%		
CD-cont.	2%		
GA-cont.	2%		
Starting material Početni materijal	41		
Required amount Tražena količina	125000		
CYCLE	WEEK	AMOUNT	ACTIVITY
I	21 2002	41	ca
II	25 2002	99	ca
III	29 2002	243	ca
IV	33 2002	596	ca
V	37 2002	1460	ca
VI	41 2002	3576	ca
VII	45 2002	8761	ca
VIII	49 2002	21464	ca
IX	01 2003	52588	ca
X	05 2003	51536	ca/cd
XI	09 2003	127551	ga
XII	12 2003	125000	la

Astrantia's species are clump forming perennials and they will thrive in a woodland garden, on a stream bank or in a moist border. They grow in a moist, fertile, preferably humus-rich soil in sun or partial shade.

Table 6 can explain micropropagation of *Astrantia* through 14 cycles.

Table no.6. Increasing plantlets number of *Astrantia* till reaching ordered amount
Tabela br.6. Povećanje broja plantleta *Astrantia-e* do postizanja naručene količine.

CA-pf	2	CA-pf - propagation factor in multiplication phase CA-pf - propagacijski faktor u fazi multiplikacije GA-pf - propagation factor for rooting phase GA-pf - propagacijski faktor u fazi zakorjenjivanja CA-cont. - contamination in multiplication phase CA- cont. - kontaminacija u fazi multiplikacije CD-cont. - contamination in phase before rooting CD-cont. - kontaminacija u fazi prije zakorjenjivanja GA-cont. - contamination in rooting phase GA- cont. - kontaminacija u fazi zakorjenjivanja	
GA-pf	1,9		
CA-cont.	1%		
CD-cont.	1%		
GA-cont.	1%		
Starting material Početni materijal	48		
Required amount Tražena količina	143300		
CYCLE	WEEK	AMOUNT	ACTIVITY
I	6 2002	48	ca
II	10 2002	94	ca
III	14 2002	184	ca
IV	18 2002	361	ca
V	22 2002	707	ca
VI	26 2002	1385	ca
VII	30 2002	2715	ca
VIII	34 2002	5321	ca
IX	38 2002	10429	ca
X	42 2002	20440	ca
XI	46 2002	40063	ca
XII	50 2002	78523	ca
XIII	02 2003	76952	ca/cd
XIV	06 2003	144747	ga
XV	10 2003	143300	la

CONCLUSIONS

1. Advantages of micropropagation compared with macropropagation are: a large number of plants achieved quickly, high health status, year-round

nursery production, long term storage of disease free stock, effective with difficult subjects made this technique useful in solving many problems of horticultural growers.

2. Economically, micropropagated plants cannot compete with conventional seed raised material but an increase in costs would be permitted if these plants were made attractive by improved quality, reliability or health status.

3. Micropropagation is tissue culture technique that has become an important part of commercial propagation for many horticultural plants approved through work of many production tissue culture laboratories such is SBW International BV.

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