Taylor & Francis
Taylor & Francis Group

Biotechnology & Biotechnological Equipment

ISSN: 1310-2818 (Print) 1314-3530 (Online) Journal homepage: https://www.tandfonline.com/loi/tbeq20

Sugar Beet Micropropagation

S. Mezei, L. Kovacev & N. Nagl

To cite this article: S. Mezei, L. Kovacev & N. Nagl (2006) Sugar Beet Micropropagation, Biotechnology & Biotechnological Equipment, 20:1, 9-14, DOI: 10.1080/13102818.2006.10817296

To link to this article: https://doi.org/10.1080/13102818.2006.10817296

SUGAR BEET MICROPROPAGATION

S. Mezei, L. Kovacev, N. Nagl
Institute of Field and Vegetable Crops, Novi Sad, Serbia and Montenegro

ABSTRACT

Vegetative in vitro multiplication is one of the most efficient methods for sugar beet (Beta vulgaris L.) propagation. The usual steps in this procedure are sterilization of explant, multiplication, rhizogenesis and acclimatization. In the paper is presented development of regeneration and multiplication techniques from different explants. It gives a detailed description of further micropropagation steps and presents necessary conditions for their realization. The paper also discuss different ways of micropropagation application especially in sugar beet breeding, but in other plant sciences as well.

Introduction

Micropropagation, i.e. vegetative multiplication in *in vitro* conditions is widely used method for sugar beet propagation. Although species from genus *Beta* do not have ability for natural vegetative multiplication, sugar beet can be propagated *in vivo* by inducing development of axillary (and sometimes also adventitious) buds (12, 42). Unfortunately, number of buds induced in this manner is quite random and very low (31), and therefore could not be widely used. All these facts resulted in development of micropropagation as most useful and efficient way for vegetative multiplication of sugar beet.

Process of micropropagation, regardless of starting explant type, has four stages: 1) sterilization of start explant and placing on nutrient medium for micropropagation, 2) multiplication, 3) rhizogenesis (root induction) and 4) acclimatization.

At the beginning, sterilization of different plant parts presented a serious problem, so for starting explants were used sterile seedlings, from which cotyledons and hypocotyl could be cut off (48). As the sterilization procedures started to improve, parts of the leaves grown in the greenhouse and *in vitro* (39, 40) were used as starting explants. With the further development of sterilization techniques came the possibilities to use other starting explants, deriving from the plants grown in fields. In time, sugar beet micropropagation was improved by using wide range of plant parts as start explants. Nowdays, it is possible to multiply sugar beet from all its vegetative and generative organs. Very efficient techniques were developed for hypocotyl, cotyledons, epicotyl, leaf, leaf stalk and dormant shoots. The best results were obtained with flower buds, inflorescences and its parts as well as flower stalks (1, 19, 43). The highest morphogenetic potential for micropropagation was detected in upper parts of inflorescence (**Fig. 1a**) in earlier stages of ontogenesis (VII and VIII stage) (3, 22), which indicated that regeneration ability depended on topographic position of explant.

Composition of mineral elements in the medium for growing plants by micropropagation *in vitro* is of great importance for the success of this method. Mineral elements are important for the growth of the explants used for micropropagation, both with regard to the forms of ions and their concentration in the medium (41). For micropropagation, in general are always used media with higher content of macro- and micro-elements, like MS (33) or B_5-(6). In sugar beet micropropagation is usually used MS medium supplemented with vita-

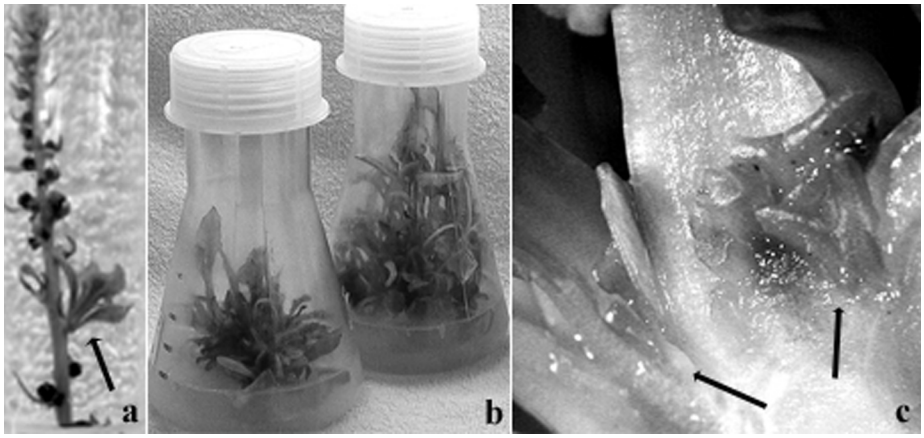

Fig. 1. Micropropagation via axillary buds:
a) induction of axillary shoots on inflorescence, b) micropropagation, c) axillary buds.

mins, sucrose and phytohormones, where ratio of auxines and citokinins plays the most important role in shoot formation. To induce and stimulate development of axillary buds usually is used citokinin, mostly benzyladenine (BA) or benzylaminopurine (BAP), combined with low concentration of giberrelic acid (2).

In the most cases sugar beet can be multiplied *in vitro* by micropropagation or somatic organogenesis. Somatic organogenesis is formation of adventitious buds from group of cells and it can be direct, from differentiated plant tissues or indirect, from dedifferentiated callus cells. In that way plants were regenerated from different explants like leaf blade, leaf stalk, leaf nerve and apical meristem (21, 50). Indirect organogenesis was obtained on calli deriving from sugar beet stalk (49), embryo (37), hypocotyl (13), cotyledon (14) and leaf (53). Regardless of starting explant and regeneration protocol used, this type of sugar beet regeneration and multiplication is strictly dependant on interaction of genotype and concentrations of phytohormones in nutrient medium.

Unlike somatic organogenesis, which is mostly used in research purposes, micropropagation by stimulation of axillary buds is used for large scale sugar beet multiplication (**Fig. 1b,c**). This way of multiplication is based on ability of sugar beet to form axillary buds when placed on medium with high concentrations of citokinins (47). The main advantage of micropropagation is the fact that ability to produce axillary buds does not depend on genotype, although it was noticed that micropropagation rate, i.e. number of produced plants can be genotype dependant (23, 32).

In order to stimulate rhizogenesis shoots are put on medium with reduced amount of nutrients (1/3 full MS), and this step usually does not present any major problem (**Fig. 2**). Since plantlets in *in vitro* culture grow under very specific conditions with high humidity, relatively low irradiance with easily available water, sugar and nutrients in presence of growth regulators, they have to go through acclimatization. Even a plants with well developed root can wilt as water loss of their leaves is not restricted (38). Therefore, the main principle of acclimatization of sugar beet from *in vitro* culture is putting it under conditions where air humidity can be gradually lowered (9). It is usually done in following way: The rooted plants are transferred to jiffy pots and put for one week in the growth chamber where humidity is gradually reduced (**Fig. 3**). Samples are then placed on

Fig. 2. Rhizogenesis and acclimatization (**Fig. 3**) of micropropagated plants.

the room temperature for one week and after that transferred to the greenhouse, where acclimatization lasts up to two months.

Application in plant breeding

Application of multiplication methods in plant breeding depends on the aim of specific programs: preservation of existing genetic variability in unchanged state, or development of new genetic variability.

Vegetative propagation via axillary buds (micropropagation) is usually used in the cases when there is necessary it preserve selected genotypes unchanged for certain period of time. It is useful in breeding programs for preservation of heterozygous genotypes during the cycle in recurrent selection program, and especially in development of improved populations of tetraploid pollinators (29). Most of European sugar beet hybrids are actually triploid hybrid varieties made by crossing diploid monogerm homozygous line with tetraploid multigerm population. Since tetraploid father component is donor of two thirds of commercial hybrid, accumulation of superior genotypes in its population is of highest importance. Beside resistance or tolerance to unfavorable conditions or pathogen attack, one of the most important traits that tetraploids are supposed to posses are good combining abilities. Determination of combining abilities in selected teraploid genotypes is usually tested during a cycle in recurrent selection, that lasts three years. In the first year, the selected tetraploid plants are crossed and their inflorescence tips are put in *in vitro* culture in order to be preserved during the selection cycle, since they are autosterile. After two years of field trials evaluation, genotypes with good combining abilities are after multiplication and acclimatization used to create new , improved population.

Vegetative propagation via adventitious buds is used when the aim of research is to create new genetic variability, i.e. to induce development of somaclonal variability. Somaclonal variability can be defined as genetic variability that occurs in *in vitro* conditions, usually in callus cells, call suspensions and in plant regenerating from adventitious meristems (4, 43). In sugar beet regenerant deriving from leaf stalks were detected changes in the leaf morphology (8), while the changes in chromosome structure and number were detected in hypocotyl regenerants (24) and in plants regenerating from the callus via organogenesis (44, 53). Increased genetic variability obtained in this might prove useful in broadening of gene pool, which could be very significant in the breeding of crops with narrow genetic background such as sugar beet.

Production of dihaploid lines Since early 80's significant progress in production of homozygous lines, has been made by use of in vitro produced sugar beet haploids and dihaploids. Haploids are obtained by ovule culture from unpollinated ovules via process called gynogenesis (10, 52) Percent of haploid induction varies from 0% up to 35%, which strictly depends from genotype and its interaction with amount of citokinins in nutrient medium (51). Obtained haploids can also be multiplied via axillary bud induction and although all genotypes have ability to multiply, after prolonged time in micropropagation there can be detected differences in micropropagation rates (35). One of the very efficient methods for sugar beet dihapliod induction is short-term micropropagation of haploids on medium with colchicine and later cytological selection of autodiploids (25, 30). The obtained lines are homozygous and therefore potentially very useful in further sugar beet breeding programs (26).

Application in other plant science research

Micropropagation is widely used method for different aspects of sugar beet research, like physiological studies of *in vitro* morphogenesis, plant growth regulation, stress studies and genetic engineering.

The advances in tissue culture led to development of new germplasm including clones, cell lines and genetically transformed material. In order to safely preserve and multiply that type of germplasm the new discipline developed under the name ***in vitro* conservation**. Maintenance and preservation of germplasm in the field gene banks is labor-consuming and expensive. Although conservation of seeds of economically important crops have been practiced since ancient times, it suffers from severe limitations like low seed viability and heterozygosity. Therefore it was necessary to develop alternative methods, like *in vitro* preservation, which includes lower labor costs efficient multiplication, with more than 1000 plants/m^2 (11), and limits disease transfer. One of the ways to preserve germplasm *in vitro* is induction of reduced vegetative growth ("slow growth") of stored material by inducing osmotic stress, limiting the availability of carbohydrates to sub-optimal level, low temperature/dark maintenance or incorporating growth retardants in the culture. Slow growth techniques are strongly recommended for the storage of shoot cultures (5, 20, 46) and were eventually tested on sugar beet. Varying concentrations of NaCl, mannitol and reduced amount of nutrients or sugar had different effect on plant preserved in vitro. Until now, the best results are achieved in conditions with increased concentrations of NaCl which slowed down micropropagation rate but left plant undamaged and able to easily recover (34).

Genetic potential of crop plants, for yield is rarely achieved due to the limitations caused by biotic and abiotic stresses, but screening for resistance or tolerance to some of them could be relatively easily done in the *in vitro* conditions. One of the most promising drought tolerance, and although some research has been done on *in vitro* screening for tolerance to low water supply (27), the most of research is yet to be done.

Although transgenic research on sugar beet are preformed for almost two decades (16, 17, 28), there is only one transformation method, PEG transformation of stomatal guard cells (7, 45), that can be used for different genotypes and transformation vectors. Since this method is technically difficult and long lasting, there were numerous tries to transform sugar beet via some *Agrobacterium*-mediated method but, until now, they resulted in very low or non existing transformation efficiency, regardless to type of start explant (15, 18, 54). The last method presented was transformation of axillary meristem (36), based on

ability of sugar beet to micropropagate. Although it gave very low transformation efficiency and few transient transformants, this type of start explant should be considered for some future sugar beet transformation research, since the ability to develop axillary buds on medium with cytokines is not genotype specific and does not depend on ploidy level.

REFERENCES

1. **Atanasov A.I.** (1980) Z. Pflanzenzucht., **84**, 23-29.
2. **Atanasov A.I.** (1986) In: Biotechnology in Agriculture and Fortestry 1. Crops, (Y.P. Bajaj, Ed.), Springer-Verlag, Berlin. 462-470.
3. **Coumans–Gilles M., Keveres F.C., Ceulemans E., Gaspar T.** (1981) Plant Cell, Tissue and Organ culture, **1/2**, 93-101.
4. **De Klerk G.-J.** (1990) Acta Bot. Neerl., **39**, 129-144.
5. **Golmirzaie A., Toledo J.** (1997-98) CIP Program Reports, 351-356.
6. **Gamborg O.L., Miller R.A., Ojima K.** (1968) Exp. Cel., **50,** 148-156.
7. **Hall R.D., Riksen-Bruinsma T., Weyens G.J., Rosquin I.J., Denys P.N., Evans I.J., Lathouwers J.E., Lefebvre M.C., Dunwell J.M., vanTunnen A., Krens F.A.** (1996) Nature Biotechnol., **14**, 1133-1138.
8. **Harms C.T., Baktir I., Oertli I.I.** (1983) Plant Cell Tissue Organ Culture, **2**, 93-102.
9. **Hazarika B.N.** (2003) Current Science, **85**(12), 1704-1712.
10. **Hosemans D., Bosseutrot D.** (1983) Z. Pflanzenzüchtg, **91**, 74-77.
11. **Hu C.Y., Wang P.J.** (1983) In: Handbook of Plant Cell Culture, (Evans et al., Eds.), Macmillan Publishing Company, New York, 10022.
12. **Hussey G., Hepher A.** (1978) Ann. Bot., **42**, 477-479.
13. **Jacq B., Tetu T., Sangwan R.S., De Laat A., Sangwan-Norreel B.S.** (1992) Plant Cell Rep., **11**, 329-333.
14. **Jacq B., Tetu T., Sangwan R.S., De Laat A., Sangwan-Norreel B.S.** (1993) Plant Breed., **110**, 185-191.
15. **Krens F.A., Trifonova A., Keizer L.C.P., Hall R.D**. (1996) Plant Sci., **116**, 97-106.
16. **Lindsey K., Gallois P**. (1990) J. Experiment. Bot. **226**(41), 529-536.
17. **Lindsey K., Gallois P., Eady C.** (1991) Plant Tiss. Culture Manual, **B7**, 1-13.
18. **Mannerloef M., Tuvesson S., Steen P., Tenning P**. (1997) Euphytica, **94**, 83-91.
19. **Margara I**. (1970) C.R. Acad. Sci. Paris, **270,** 698-701.
20. **Matsumoto T., Sakai A.** (2003) Euphytica, **131**, 299-304.
21. **Mezei S., Kovacev L.** (1988) Genetika, **20**(2), 147-152.
22. **Mezei S., Jelaska S., Kovacev L.** (1990) Journal of Sugar Beet Research, **27**(3/4), 90-96.
23. **Mezei S., Kovacev L.** (1991) Savremena poljoprivreda, **39**(1) 53-60.
24. **Mezei S., Kovacev L.** (1992) Genetika, **24**(3) 173-179.
25. **Mezei S., Zlokolica M., Kovacev L.** (1992) Annals of Biology, **8**(2), 116-120.
26. **Mezei S., Kovacev L.** (1992) Proceedings of XIII EUCARPIA Congres, France, 191-193.
27. **Mezei S., Kupresanin N., Zlokolica M., Kovacev L., Cacic N.** (1996) Proceedings of the International Symposium Drought and Plant production. Donji Milanovac, 17-20 Sept., 93-97.
28. **Mezei S., Mrkovacki N., Nagl N., Kovačev L., Ognjanov V.** (1996) J. Sci. Agric. Research, **57**(205), 23-29.
29. **Mezei S., Cacic N., Kovacev L., Sklenar P., Nagl N.** (2000) Selekcija i Semenarsto, **VII,** 1-2, 61-65.
30. **Mezei S., Kovacev L., Cacic N., Nagl N.** (2002) Biotechnol. & Biotechnol. Eq., **16(2)**, 58-62.
31. **Miedema P., Groot P.J., Zuidgeest J.N.M.** (1980) Euphytica, **29**, 425-432.
32. **Miedema P.** (1982) Euphytica, **31**, 635-643.
33. **Murashige T., Skoog F.** (1962) Physiol. Plant., **15**, 473-497.
34. **Nagl N., Mezei S., Kovačev L., Čačić N.** (2004) Proceedings of ESNA (European Society for New Methods in Agricultural Research) XXXIV annual meeting, Novi Sad, Serbia and Montenegro, 270-273.
35. **Nagl N., Mezei S., Kovacev L., Vasic D., Cacic N.** (2004) Genetika, **36**(3), 187-194.
36. **Nagl N.** (2004) In: Transgenic resistance to beet necrotic yellow virus, Andrejevic Endowment, Belgrade, Serbia & Montenegro.
37. **Nakashima H., Tsuda C., Kudo C.** (1986) Proceedings of the Sugar Beet Research Association, 26th Research meeting of Sugar Beet Technical Cooperation, Japan, 39-43.
38. **Pospisilova J., Ticha I., Kaldecek P., Haisel D., Plzakova S.** (1999) Biologia Plantarum, **42**(4), 481-497.
39. **Rogozinska I., Goska M.** (1978) Bull. Acad. Pol. Sci., **5**, 343-345.

40. **Rogozinska I., Katowinska U., Goska M.** (1979) Hod. Ros. Akl. Nasien., **23**, p. 1.
41. **Saric M., Mezei S., Ruzic D.J.** (1995) Archives of Biological Sciences, **47**, 1-2.
42. **Saunders J.W.** (1982) Crop. Sci., **22**, 1102-1105.
43. **Saunders J.W., Mohoney M.D.** (1982) Euphytica, **31**, 801-804.
44. **Saunders J.W., Doley W.P.** (1986) J. Plant Physiol., **124**, 473-479.
45. **Sevenier R., Hall R.D., v.d.Meer I.M., Hakkert H.J.C., v.Tunnen A.J. and Koops A.J.** (1998) Nature Biotech. **16**, 843-846.
46. **Shibli R.A., Shatinawi M.A., Ajlouni M.M., Jardat A., Adham Y.** (1999) Adv. Hort. Sci., **13**, 133-134.
47. **Slavova V.I.** (1980) Fiziologija na rastenijata, **VI**, **1**, 35-39 (Bg.).
48. **Slavova V.I.** (1980) Fiziologija na rastenijata, **VI**, **2**, 83-88 (Bg.).
49. **Slavova I.** (1981) Fiziologija na rastenijata **VII 4,** 71-77 (Bg.).
50. **Slavova I., Zahariev A., Ivanova G.** (1982) Fiziologija na rastenijata, VIII **1**, 95-99 (Bg.).
51. **Slavova V.I.** (1993) Biotechnol. & Biotechnol. Eq., **7(2)**,34-35.
52. **Van Geyt J.P.C., Speckmann Jr., D'Haullin K., Jacobs M.** (1987) Theor. Appl. Genet., **73**, 920-925.
53. **Yu M.H.** (1989) Crop Sci., **29**, 205-209.
54. **Zhang C.-L., Chen D.-F., Kubis S., McCormac A., Kubalakova M., Zhang J., Bao M.-Z., Scott N.W., Slater A., Heslop-Harrison J.S., Elliot M.C.** (1998) Proc. 61th IIRB Congress, Brussels, Belgium, 381-389.