Phyton (Austria) Special issue: "Plant Physiology"	Vol. 39	Fasc. 3	(283)-(287)	30. 11. 1999

Studies of Somaclonal Variation in Hop (*Humulus lupulus* L.)

By

Jelka ŠUŠTAR - VOZLIČ[1)], Branka JAVORNIK[2)] & Borut BOHANEC[2)]

Key words: Hop, breeding, somaclonal variation, RAPD, flow cytometry.

Summary

ŠUŠTAR–VOZLIČ J., JAVORNIK B. & BOHANEC B. 1999. Studies of somaclonal variation in hop (*Humulus lupulus* L.) - Phyton (Horn, Austria) 39 (3): (283) - (287).

Various combinations of auxins and cytokinins were tested to achieve adventitious bud induction from hop callus cultures induced on stem or petiole explants of 16 genotypes and regenerants were scored to establish the occurence of genetic changes. A total of 136 plants from 10 cultivars were regenerated. The most important factors in the regeneration process were a single step callus induction and a shoot regeneration protocol without subculturing, while the results from various media composition showed that in the presence of 0.29 or 1.43 μM IAA, cytokinins are essential in the induction process. For a specific genotype 5.69 μM zeatin riboside, 9.12 μM zeatin or 23.20 μM kinetin was optimal. Regeneration ability varied among genotypes from 0 to 37.5% and was highest in the genotype 200/69. The regenerants obtained were tested with RAPD analysis and flow cytometry for possible genetic changes. RAPD profiles did not differ from donor cultivars in 43 regenerants tested using 22 decamer primers. Flow cytometric analysis showed that 4 out of 46 regenerants were tetraploid while the others remained diploid.

Introduction

Hop is a dioecious perennial plant species that is grown for its ripe female inflorescence; offspring obtained by crossings are highly heterozygous. Somaclonal variation can be applied as a source of genetic changes in tissue culture in order to shorten the breeding process in already established hop cultivars, when improvement of only a small number of traits is desired.

[1)] Agricultural Institute of Slovenia, Hacquetova 17, 1001 Ljubljana, Slovenia.

[2)] Biotechnical Faculty, Agronomy Dept., Centre for Plant Biotechnology and Breeding, Jamnikarjeva 101, 1111 Ljubljana, Slovenia.

No hop cultivar has up to now been obtained by means of inducing somaclonal variation. Only HEALE & al. 1989 have reported obtaining some variations in specific agronomic traits, but somaclonal variation in regenerants from green callus did not lead to the selection of stable, wilt-resistant lines in the field. A relatively high percentage of adventitious shoot regeneration has been reported in a few hop genotypes. RAKOUSKY & MATOUŠEK 1994 investigated the regeneration ability from internodal, stem and leaf explants of two Czech cultivars. Shoots were regenerated by direct organogenesis, the highest percentage of regenerants (up to 52 %) has been obtained on media supplemented with 2.22 to 8.87 µM BA. BATISTA & al. 1996 reported the regeneration of shoots from callus culture of clone 'Braganca' and cultivar 'Brewer's Gold'. On basal media (MS or SH) supplemented with 0.14 µM IAA and 8.87 µM BA, shoot regeneration was up to 72% with the clone 'Braganca', while the percentage of regenerated shoots of the cultivar 'Brewer's Gold' is reported to be much lower on all the media tested. In our preliminary study of shoot induction (ŠUŠTAR-VOZLIČ & BOHANEC 1994) which included different basal media - MS (MURASHIGE & SKOOG 1962), B5 (GAMBORG & al. 1968) and SH (SHENK & HILDEBRAND 1972), vitamins, various carbohydrates (glucose, sucrose, maltose) and light regimes we concluded that MS medium, glucose and 16/8 hour photoperiod was optimal for callus growth, while shoot regeneration was not achieved.

The nature of genetic modifications responsible for somaclonal variation has been investigated by many authors at different levels, but its molecular basis is still poorly understood. Among the various methods used for screening of somaclonal variation, the use of RAPD markers has been reported in different plant species (HASHMI & al. 1997, GODWIN & al. 1997) as well as the application of genome size measurement using flow cytometry (BOASE & HOPPING 1995, RIVAL & al. 1997).

The objectives of the research presented were to establish a protocol for adventitous shoot regeneration and to evaluate regenerants for possible somaclonal variation occuring during the process of organogenesis from undifferentiated tissue, using RAPD markers and flow cytometric analysis.

Materials and Methods

The Slovene cultivars 'Aurora', 'Bobek', 'Cerera' and 'Savinjski golding', together with foreign cultivars 'Spalter', 'Cascade', 'Willamette', 'Wye Northdown', 'Galena', 'Nugget', 'Brewers Gold', 'Fuggle 37', 'Zenith' and experimental hybrids 200/22, 200/69, 07P14 were studied. In vitro grown virus-free plants were used as donor plants, and in some experiments, explants from in situ grown plants were also inoculated. Stem or petiole explants were inoculated in 60 mm Petri dishes (8 per dish) on induction media and grown at 25°C at a 16/8 photoperiod. The basal medium included MS macro and micro elements, 100 mg/l myo-inositol, 0.5 mg/l niacin and pyridoxine, 0.1 mg/l thiamine and Ca-pantothenate, 20 g/l glucose, 3 g/l agar, 1 g/l gellan-gum and various growth regulators. Elongation of shoots was achieved on basal medium supplemented with 4.40 µM BA, and rooting was achieved when the basal medium was supplemented with 1.14 µM IAA.

A protocol that has been described for hops (ŠUŠTAR-VOZLIČ & JAVORNIK 1995) was used for the RAPD analysis of regenerants, using 22 decamer primers from Operon Technologies (OPA-01, OPA-04, OPA-07, OPA-09, OPA-10, OPA-11, OPA-12, OPA-17, OPB-04, OPB-07, OPB-08, OPB-10, OPB-11, OPM-02, OPM-05, OPX-01, OPX-03, OPX-04, OPX-07, OPX-08, OPX-09, OPX-11). For flow cytometry analysis, leaves were chopped with a razor blade in 1 ml of 0.1 M citric acid containing 0.5% Tween 20, and the suspension was filtered through a 50 µm nylon gauze filter. A 3-fold volume of dye solution containing 5.25 µg/ml 4',6-diamidino-2-phenylindole (DAPI) in a 0.4 M di-sodium hydrogen phosphate was added. *Trifolium repens* was prepared in the same way and was used as internal standard.

Results and Discussion

Shoots were induced from stem explants 30 to 120 days days after inoculation. Individual shoots subcultured on elongation and rooting media developed into rooted plantlets within 6 weeks. The regeneration ability was genotype dependent, and the impact of the type and concentration of growth regulators was established (Table 1). The highest percentage of explants with regeneration (37.5%) was achieved with accession 200/69 on basal medium supplemented with 23.20 µM kinetin and 0.29 µM IAA. For the cultivar 'Spalter', the percentage of explants with regeneration was 31.1% on medium supplemented with 9.12 µM zeatin and 0.29 µM IAA. Cultivar 'Savinjski golding' gave the highest percentage of regenerants on medium supplemented with 23.20 µM kinetin and 1.43 µM IAA (29.2%), or on medium with 5.69 µM zeatin riboside and 0.29 µM IAA (26.6%). No regenerants were obtained for the cultivars 'Bobek', 'Cerera', 'Willamette', 'Wye Northdown', 'Fuggle 37' and 'Galena', although several experiments were performed and a number of explants were inoculated.

In the previous research, mainly reported in ŠUŠTAR-VOZLIČ & BOHANEC 1994, no induction of buds was achieved, although a number of different media compositions were tested, various treatments were applied and a relatively large number of explants were inoculated. Several regenerants were obtained by altering the induction protocol (mainly by omitting subculturing). It was additionally shown that the explants with the best regeneration ability were stem cuttings originating from newly established shoot cultures, while the optimal media contained a relatively high cytokinin concentration. Differences in percentage of explants with regeneration were very high and varied from 1.4 to 37.5% (Table 1). Six out of 16 cultivars produced no regenerants.

Forty-three regenerants - 26 from the cultivar 'Savinjski golding', 8 from 'Spalter', 4 from 200/69, 2 from 200/22 and 'Aurora', and 1 regenerant from the cultivar 'Cascade', were analysed using molecular markers for detection of minor genetic changes. RAPD analysis was performed using 22 selected polymorphic primers producing a total of 165 scorable bands. Genetic analysis revealed no differences in RAPD patterns in comparison to the donor cultivars. The analysis of genome size using flow cytometric measurement of 46 regenerants revealed genome doubling to tetraploid level in 4 out of 46 regenerants tested. Such major genetic changes have been reported as the result of shoot regeneration process in

several other species, such as *Actinidia deliciosa* (BOASE & HOPPING 1995) and *Saintpaulia ionantha* (WINKELMANN & GRUNEVALDT 1995).

On the basis of the results presented, we may conclude that adventitious shoot regeneration in hop does lead to some level of genetic changes, although the desired small changes, occuring on the molecular level, were not found. Taking into account the relatively small fraction of genome scored by RAPD analysis, we cannot exclude that such changes were induced but were not detected. The regenerated plants have been planted in soil in order to evaluate possible changes of morphological and chemical properties in comparison to the original cultivars.

Table 1. Impact of the type and concentration of growth regulators added to basal media on the percentage of stem explants with regeneration for individual genotypes, growth regulators, and number of explants inoculated for individual experiment.

Genotype	Growth regulators (μM)	No. of explants	% of explants with regeneration
Spalter	zeatin (9.12) + IAA (0.29)	88	31.1
200/69		80	12.5
Cascade	zeatin (9.12) + IAA (1.43)	64	1.6
Brewer's Gold		64	1.6
200/69		48	4.2
Savinjski golding	zeatin riboside (5.69) + IAA (0.29)	256	26.6
Spalter		136	12.5
Cascade		176	2.1
200/69		256	12.5
200/22		184	1.4
Savinjski golding	zeatin riboside (5.69) + IAA (1.43)	208	10.4
Aurora	kinetin (23.20) + IAA (0.29)	232	12.5
Savinjski golding		264	6.3
Spalter		16	12.5
200/69		72	37.5
200/22		168	9.4
07P14		16	12.5
Savinjski golding	kinetin (23.20) + IAA (1.43)	304	29.2
Aurora		240	6.3
Cascade		96	1.6
200/69		48	6.3
200/22		96	2.1
Brewer's Gold	TDZ (4.54) + IAA (1.43)	48	2.1
Zenith		48	2.1
Savinjski golding	TDZ (9.08) + IAA (0.29)	208	8.3
Savinjski golding	TDZ (9.08) + IAA (1.43)	208	20.8
Cascade		64	1.6
Savinjski golding	BA (8.87)	96	2.1
Savinjski golding	BA (8.87) + IAA (1.43)	48	2.1
Cascade		48	2.1
Nugget		48	2.1
Zenith		48	6.3
200/69		48	4.2

References

BATISTA D., SOUSA M.J. & PAIS M.S. 1996. Plant regeneration from stem and petiole-derived callus of *Humulus lupulus* L. (hop) clone Bragança and var. Brewer's Gold. - In Vitro Cell. Dev. Biol. 32: 37-41.

BOASE M. R. & HOPPING M. E. 1995. DNA dodecaploid plants detected among somaclones of *Actinidia deliciosa* var. *deliciosa* cv. Hayward. - Plant Cell Rep. 14: 319-323.

GAMBORG O.L., MILLER R.A. & OJIMA K. 1968. Nutrient requirements of suspension cultures of soybean root cells. - Experimental Cell Res. 50: 151-158.

GODWIN I.D., SANGDUEN N., KUNANUVATCHAIDACH R., PIPERIDIS G. & ADKINS S.W. 1997. RAPD polymorphisms among variant and phenotypically normal rice (*Oryza sativa* var. *indica*) somaclonal progenies. - Plant Cell Rep. 16: 320-324.

HASHMI G., HUETTEL R., MEYER R., KRUSBERG L., HAMMERSCHLAG F. 1997. RAPD analysis of somaclonal variants derived from embryo callus cultures of peach. - Plant Cell Rep. 16: 624-627.

HEALE J.B., LEGG T. & CONNELL S.A. 1989. *Humulus lupulus* L. (hop): In vitro culture; Attempted production of bittering components and novell disease resistance. - In: BAJAJ Y. P. S. (Ed.), Biotechnology in agriculture and forestry, Vol. 7, Medicinal and aromatic plants, pp. 264-285. – Springer Verlag, Berlin, Heidelberg.

MURASHIGE T. & SKOOG F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. - Physiologia Plantarum 15: 473-497.

RAKOUSKY S. & MATOUŠEK J. 1994. Direct organogenesis in hop - a prerequisite for an application of *A. tumefaciens*-mediated transformation. - Biologia Plantarum 36: 191-200.

RIVAL A., BEULE T., BARRE P., HAMON S., DUVAL Y., NOIROT M. 1997. Comparative flow cytometric estimation of nuclear DNA content in oil palm (*Elaeis guineensis* Jacq) tissue cultures and seed -derived plants. - Plant Cell Rep. 16: 884-887.

SHENK R.U. & HILDEBRANDT A.C. 1972. Medium and techniques for induction and growth of monocotyledonous and dicotyledonous plant cell cultures. - Can. J. Bot. 50: 199-204.

ŠUŠTAR-VOZLIČ J. & BOHANEC B. 1994. Hop callus culture and attempts of shoot regeneration. – In: JAVORNIK B., BOHANEC B. & KREFT I. (Eds.), Proc. of the int. colloq. impact of plant biotechnol. on agric., Rogla, Slovenia, Dec 5-7 1994, pp. 57-63

— & — 1995. Use of molecular markers in hops (*Humulus lupulus* L.). - In: SEIGNER E. (Ed.), Proc. of the scientific commission of international hop growers convention pp. 32-39. - Strasbourg, France, Jul 25-28 1995.

WINKELMANN T. & GRUNEWALDT J. 1995. Analysis of protoplast-derived plants of *Saintpaulia ionantha* H. Wendl. - Plant Breed. 114: 346-350.