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ANGELINA SUBOTIĆ, LJILJANA RADOJEVIĆ

PLANT REGENERATION OF *IRIS HALOPHILA* PALL. AND *IRIS SIBIRICA* FANCH. BY SOMATIC EMBRYOGENESIS AND ORGANOGENESIS

Institute for Biological Research „Siniša Stanković", Belgrade, Yugoslavia

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Plant regeneration was achieved by somatic embryogenesis and organogenesis in *Iris halophila* Pall. and *Iris sibirica* Fanch. Somatic embryogenesis was induced after seven days by growing emryo explants on B_0 nutrient medium based on MS supplemented with (in mg/l): 2,4-D 5.0, KN 1.0, CH 250.0 and Pro 250.0. Further development of embryogenic callus and differentiation of somatic embryos occurred on B_5 nutrient medium (MS, 2,4-D and KN, 1.0 mg/l, each). The embryogenic potential depended on both the species and the composition of the nutrient medium. For *I. halophila* on B_5 nutrient medium it was 79% and for *I. sibirica* on B_1 it went up to 57%. In addition to NEC, EC and OC also formed on the both nutrient media. During the OC growth of both species on C_3 nutrient medium consisting of MS, BAP and GA_3 (1.0 and 0.1 mg/l, respectively) adventitious buds were induced. Shoot multiplication in *I. halophila* (89%) and *I. sibirica* (98%) was achieved on C_2 medium supplemented with BAP and NAA (1.0 and 0.1 mg/l, respectively). Somatic embryos germination and shoot rooting was attained in MS mineral

solution containing KN 3.0, IAA 1.0 and GA_3 0.1 (mg/l). Regenerants of both species were successfully acclimated under greenhouse conditions.

Key words: *Iris halophila* Pall., *Iris sibirica* Fanch., adventitious buds, embryogenic callus, embryo culture, organogenesis, somatic embryogenesis, somatic embryos.

Ključne reči: *Iris halophila* Pall, *Iris sibirica* Fanch, adventivni pupoljci, embriogeni kalus, kultura embriona, organogeneza, somatska embriogeneza, somatski embrioni.

Abbreviations: AB - adventitious buds; AS - adenine sulphate; CH - casein hydrolysate; 2,4-D - dichlorophenoxyacetic acid; IAA - indole-3-acetic acid; IBA - 2-indolebutyric acid; KN - 6 - furfurylaminopurine; BAP - 6-benzylaminopurine; GA_3 - gibberellic acid; MS - Murashige and Skoog mineral solution; NAA - α-naphtaleneacetic acid; NEC - nonembryogenic callus; EC - embryogenic callus; OC - organogenic callus; Pro - l-proline; SE - somatic embryos; Tyr-l-tyrosine.

INTRODUCTION

The Iris genus includes a great number of species, many of them being important for horticulture and pharmaceutical industry. Similar to most of monocot plants with rhizomes, irises are propagated vegetatively, each individual producing a maximum of some ten plants per year. During the last twenty years *in vitro* propagation of the species was intensified in different ways: using callus in *I. hollandica* (Hussey, 1976), regneration from callus and somatic embryogenesis in *Iris spp.* (Meyer, Fuchigami & Roberts, 1975; Reuther, 1977), somatic embryogenesis by *in vitro* culture of mature *I. pumila* and *I. setosa* embryos (Radojević, Sokić & Tucić, 1987; Radojević & Subotić, 1992), root culture in *I. pseudocorus, I. setosa* and *I. versicolor* (Laublin, 1991) and leaf culture and immature inflorescence culture in *I. pallida* and *I. germanica* (Jéhan et al., 1994).

In the present work the effect of nutrient media composition, hormones and/or amino acids on plant regeneration through somatic embryogenesis and organogenesis were studied using explants from mature zygotic embryos of *I. halophila* and *I. sibirica*.

MATERIALS AND METHODS

Seeds of *I. halophila* Pall (2n = 44) and *I. sibirica* Fanch. (2n = 28) were obtained from the collection of the Moscow Botanical garden. Sterilization and isolation of seeds were performed as described previously (Radojević & Subotić, 1992). For induction of somatic embryogenesis MS solution containing sucrose 5%, agar 0.5% and (in mg/l): inositol 100.0, Pro 250.0, CH 250.0, nicotinic acid 5.0, pantothenic acid 10.0, B_1 vitamin 2.0, 2,4-D 5.0 and KN 1.0 (B_0) was employed. Nutrient media for embryogenic callus (EC) development and somatic embryos (SE) differentiation (B_1 - B_5) are listed in Table 1. For organogenic callus (OC) and adventitious bud (AB) induction and shoot multiplication, (C_0 - C_3) were applied (Table 1). Rooting of the shoots was achieved in MS liquid medium supplemented by IAA (1.0 mg/l) and KN (3.0 mg/l). Cultures were grown at 25 ± 2 C°, 16h / 8h photoperiod (fluorescent lamps "Tesla", Pančevo, 65W, 4500 K) and regenerants rooted. Plantlets were grown in a mixture of sand and soil (3 : 1) in a greenhouse.

Tab. 1. – Composition of nutrient media for regeneration of Iris halophila and I. sibirica

Nutrient media (ingredients in mg/l)
B_0 : MS + 2,4 - D 5.0 + KN 1.0 + Pro 250.0 + CH 250.0
B_1 : MS + 2,4 - D 1.0 + KN 1.0 + Pro 250.0
B_2 : MS + 2,4 - D 1.0 + KN 1.0 + Pro 250.0 + CH 25.0
B_3 : MS + 2,4 - D 1.0 + KN 1.0 + Pro 25.0 + CH 250.0
B_4 : MS + 2,4 - D 1.0 + KN 1.0 + CH 250.0
B_5 : MS + 2,4 - D 1.0 + KN 1.0 + Pro 250.0 + CH 250.0
C_0 : MS + IAA 0.1 + BAP 1.0 + Tyr 100.0 + AS 80.0
C_1 : MS + IBA 0.1 + BAP 1.0 + Tyr 100.0 + AS 80.0
C_2 : MS + NAA 0.1 + BAP 1.0 + Tyr 100.0 + AS 80.0
C_3 : MS + GA_3 BAP 1.0 + Tyr 100.0 + AS 80.0

RESULTS

Somatic embryogenesis

Mature zygotic embryos of *I. halophila* and *I. sibirica* after seven days of growth on B_0 medium for somatic embryogenesis induction, expressed a different morphogenetic potential. During this period, tiny, yellow-pale structures appeared in *I. halophila*, while no callus or nodules were formed on zygotic embryos of *I. sibirica*. After three weeks, explants of both species grown on B_5 nutrient medium with reduced concentration of 2,4-D from 5.0 to 1.0 mg/l developed three types of calli as a result of different differentiation pathways: NEC, EC, OC (Fig. 3. a and b). In embryogenic cultures of both species, the number of SE was increasing parallel to the duration of subculture. Somatic embryos were differentiated mainly at the surface of embryogenic nodules representing a part of EC or, rarely, they were developed adventitiously at the already present SE. In most *I. halophila* cultures SE were developed to globular and heart-shaped stages and there were far less embryos with lateral scutellar notches and in the coleoptile stage (Fig. 3. c). In certain cultures of *I. halophila* grown on B_5 nutrient medium, a simultaneous maturation and germination of SE into plantlets occurred. During a prolonged growth the roots were spontaneously developed.

The highest embryogenic response was obtained in *I. halophila* grown on B_5 medium and in *I. sibirica* grown on B_1 medium (Fig. 1, values represent the means ± S.E. of results from three replicates). In order to increase the biomass of EC and number of SE, embryogenic calli were further cultured on B_0 - B_5 nutrient media. The highest values for fresh callus mass of both iris species from approx. 1600 to 2500 mg, were obtained by growing the EC on B_2 medium.

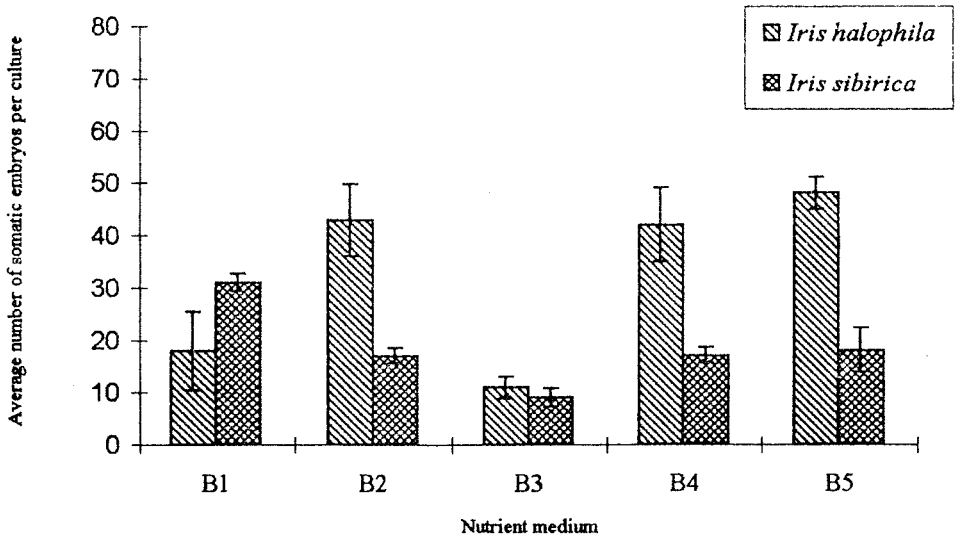

Fig. 1. – Influence of nutrient composition of somatic embriogenesis in *Iris halophila* and *I. sibirica*

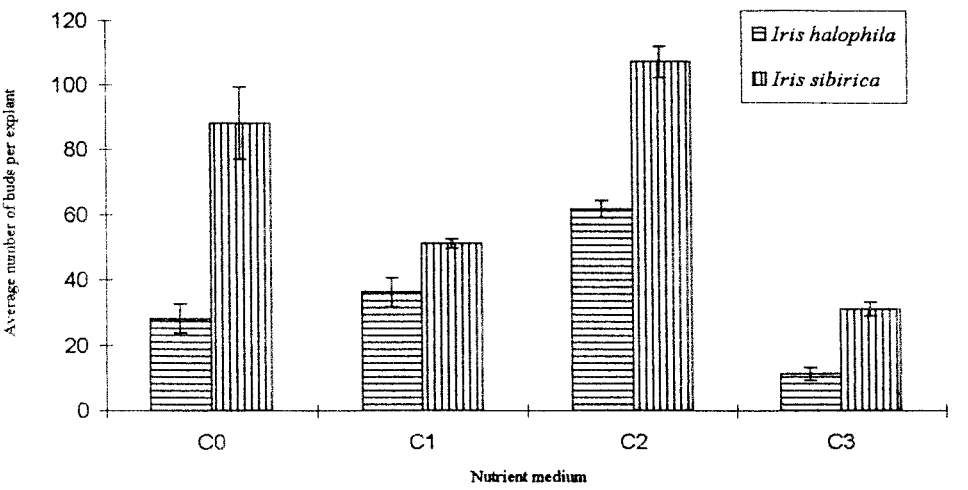

Fig. 2. – The effect of nutrient medium composition on adventitious bud multiplication of *Iris halophila* and *I. sibirica*

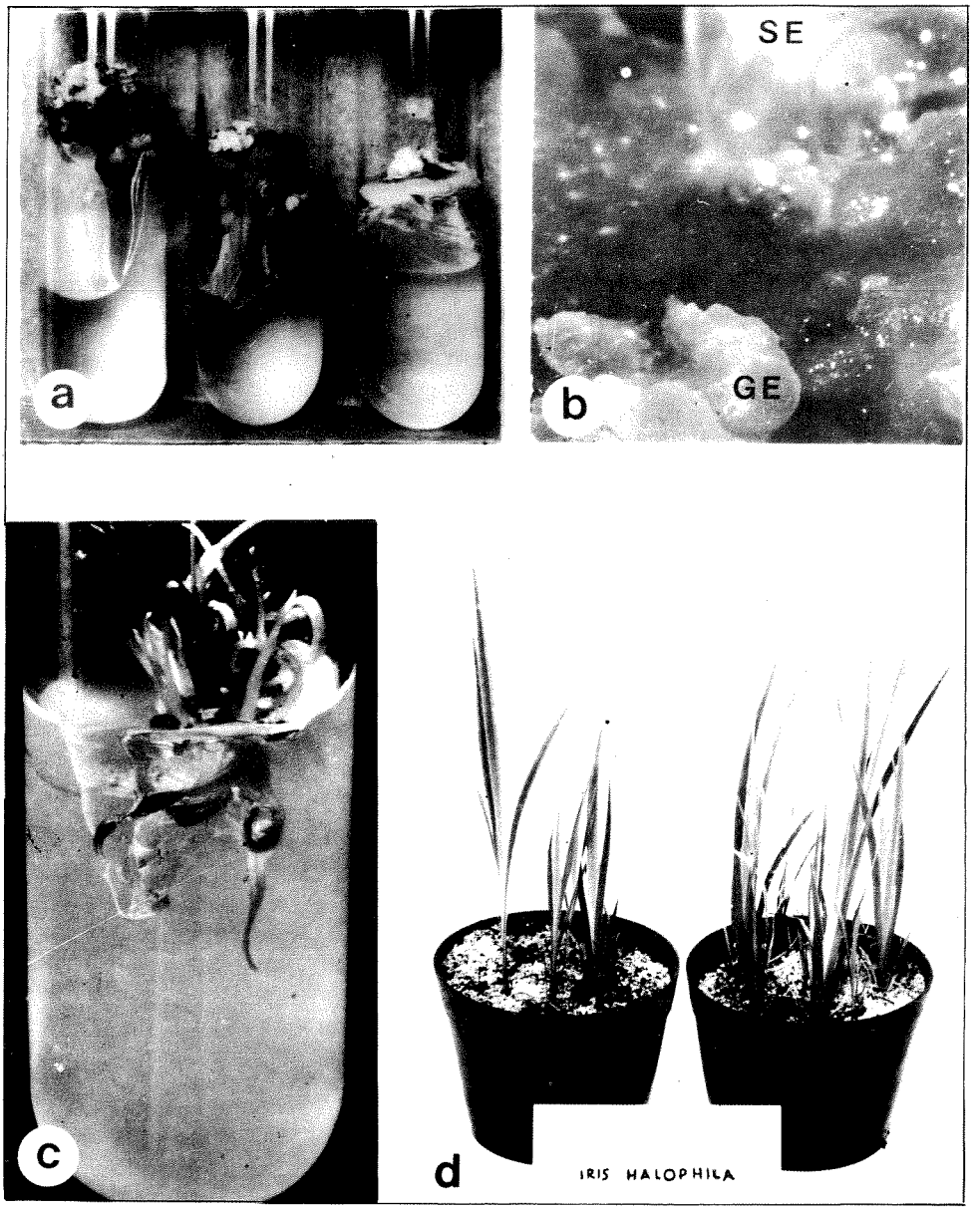

Fig. 3. – Somatic embryogenesis and organogenesis in the culture of zygotic *I. halophila* and *I. sibirica* embryos; a) – Cultures of *I. halophila* and *I. sibirica* on B_5 nutrient medium after weeks of culture; b) – EC of *I. halophila* (a detail) in an early (GE) and a late (SE) stage of development; c) – Multiplication of *I. halophila* after three subcultures; d) – Acclimatized *I. halophila* plants.

Organogenesis

Organogenesis in *I. halophila* and *I. sibirica* species was expressed after preculture of mature zygotic embryos and their growth on B_0 medium with a high concentration of 2,4-D and KN. Green organogenic callus of both iris species was observed already within the first three weeks of culture on C_3 medium. During furhter subcultures, on the same medium, AB (Fig. 3, d) was developed from OC. In order to get a higher number of regenerants the influence of different media (C_0-C_3) on the AB development was examined (Fig. 2). Most of *I. halophila* shoots were developed on C_0 nutrient medium. In *I. sibirica*, the highest number of AB, and thus the highest multiplication was achieved on C_2 medium. The lowest values of multiplication for both iris species were obtained when grown on C_3 nutrient medium. Number of rooted shoots grown in a D nutrient medium was found to be 92% and 96% for *I. halophila* and *I. sibirica*, respectively. Acclimation of plants was about the same for both iris species (Fig. 3, e).

DISCUSSION AND CONCLUSION

Regeneration of plants was achieved by somatic embryogenesis and organogenesis of the two iris species by the culture of mature zygotic embryos. For induction of both processes the presence of high auxin concentration, (2,4-D 5.0 mg/l) is required. The stage of induction in the culture of zygotic embryos is necessary for expression of morphogenetic potential is previously shown for *I. pumila* and *I. setosa* (Radojević et al., 1987; Radojević & Subotić, 1992). A decrease of auxin concentration to 0.1 mg/l in the cultures of the two iris species led to differentiation of three calli types, NEC, EC and OC. Isolation and separation of these calli enabled two pathways of regeneration to proceed. Medium containing 2,4-D and KN (1.0 mg/l) and supplemented with 250.0 mg/l of each CH and Pro (B_5) was found to be the most suitable for furhter EC growth and SE differentiation. Stimulatory action of Pro on somatic embryogenesis has been observed previously in maize (Armstrong & Green, 1985) and in different *Iris spp.* (Radojević et al., 1987, 1992; Jehan, 1994).

For the initial stages of the OC growth and the AB differentiation, C_3 nutrient medium was the most convenient. The best shoots multiplication of the both iris species was obtained on C_2 medium supplemented with BAP and NAA. These results are in accordance with the data of Hussey (1976) who obtained 50 to 100 shoots from a single initial explant of *Iris spp.* It is obvious that different growth regulators influence prominently the number of shoot produced per culture. Germination of somatic embryos and rooting of the AB was successfully achieved in D liquid nutrient medium with KN, IAA and GA_3 as previously described for *I. setosa* (Radojević & Subotić, 1992). This protocol for plant regeneration of both *Iris* species, through somatic embryogenesis and organogenesis, could be used for large scale production of regenerants.

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Rezime

ANGELINA SUBOTIĆ, LJILJANA RADOJEVIĆ

REGENERACIJA BILJAKA *IRIS HALOPHILA* PALL. I. *IRIS SIBIRICA* FANCH. PUTEM SOMATSKE EMBRIOGENEZE I ORGANOGENEZE

Institut za biološka istraživanja „Siniša Stanković", Univerziteta u Beogradu, Beograd, Jugoslavija

U kulturi zigotskih embriona *Iris halophila* i *I. sibirica* postignuta je regeneracija biljaka procesom somatske embriogeneze i organogeneze. Sukcesivnim delovanjem hranljivih podloga, sa različitim auksinima i citokininima, indukovani su neembriogeni (NEC), embriogeni (EC) i organogeni (OC) kalusi, a zatim somatski embrioni (SE) i adventivni pupoljci (AB). Somatska embriogeneza je indukovana na MS mineralnom rastvoru sa (u mg/l): 2,4-D 5.0, KN 1.0, CH 250.0 i Pro 250.0 (podloga B_0). Posle sedam dana gajenja na indukcionoj podlozi eksplantati su preneti na podlogu za diferencijaciju i razviće somatskih embriona, sa smanjenom koncentracijom 2,4-D 1.0 mg/l (podloga B_5). Na ovoj podlozi posle 2-3 nedelje gajenja pojavili su se somatski embrioni na svim razvojnim stadijumima. Razviće organogenog kalusa dobijeno je gajenjem eksplantata na C_3 hranljivoj podlozi sa (u mg/l): BAP 1.0, GA_3 0.1, Tyr 100.0 i AS 80.0. Multiplikacija izdanaka kod obe vrste najuspešnija je njihovim gajenjem na C_2 hranljivoj podlozi. Ožiljavanje zrelih somatskih embriona i izdanaka odvijalo se na MS hranljivoj podlozi sa KN 3.0, IAA 1.0 i GA_3 0.1 (mg/l). Regeneranti, obe vrste, su aklimatizovani na uslove staklare.