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IN VITRO PROPAGATION OF *JANKEA HELDREICHII* BOISS. *(GESNERIACEAE)*

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Vinterhalter, B., Vinterhalter, D. and Budimir, S. (1995): *In vitro propatation of Jankea heldreichii Boiss. (Gesneriaceae).* – Glasnik Instituta za botaniku i botaničke bašte Univerziteta u Beogradu, Tom XXIX, 129 - 135.

Shoot cultures of *Jankea heldreichii* were established from seeds aseptically germinated on hormone-free MS medium. Various explants including cotyledone and hypocotyle fragments, axillary buds and whole shoots were transfered to MS medium supplemented with 5.0 mg/l BA and 0.1 mg/l IBA for shoots induction. Shoots regenerated directly without intervening callus on types of explants. Most responsive were cotyledone fragments and whole shoots manifesting 80% and 100% shoot regeneration respectively. Shoot cultures were maintained on medium with BA decrased to 0.2-0.5 mg/l BA and rooted on medium with 0.5 mg/l IBA.

Key words: *in vitro*, propagation, shoot cultures, *Jankea heldreichii.*

Ključne reči: *in vitro,* razmnožavanje, kulture izdanaka, *Jankea heldreichii.*

INTRODUCTION

Jankea heldreichii Boiss. is an endemic and relic species of Balkan Peninsula. Nowadays it can be found only on limestone of Olympus mountain (Greece). It belongs to *Gesneriaceae* family which since tertiary is represented in Europe with only few species. Two of them *Ramonda serbica* and *R. nathalie* are native to Serbia. Family *Gesneriaceae* contains genera with species which are propagated as decorative plants. Here belong *Saintpaulia* (african violet), *Streptocarpus* and *Gloxinia*. *Jankea heldreichii* is a small rosette forming plant with thick, hairy, grey-green leaves. Investigation presented here were started wtih the general aim to develop a method suitable for vegetative propagation as an aid in protection of this species.

MATERIAL AND METHODS

Seeds of *J. heldreichii* were collected near village Petra at mountain Olympus in Greece (Stefanović et al., 1992). Seeds were surface sterilized for 30 minutes in 20% commercial bleach (containing 4-5% NaOCl) and then thoroughly rinsed in autoclaved water. Seeds were aseptically germinated on hormone free medium supplemented with 3% sucrose, 0.7% agar, MS (Murashige & Skoog, 1962) vitamins and 1/2 WPM (Lloyd & McCown, 1981) mineral medium. Preparation of medium and conditions in the growth room were same as previously described (Vinterhalter & Vinterhalter, 1994). After germination various explants including fragments of cotyledons, hypocotyls and leafs, apical buds even whole plants were transferred to medium for shoot induction. This medium supplemented with 5.0 mg/l BA and 0.1 mg/l NAA was prepared with MS or WPM salts. Shoot cultures were maintained on media supplemented with 0.1-0.5 mg/l BA and 0.05-0.1 mg/l IBA. Rooting was performed on media supplemented with 0.5 mg/l IBA. In some experiments after initial exposure to the rooting medium supplemented with IBA, shoots were transferred to hormone free medium.

For histological examination material was fixed in FAA (formalin/acetic acid / ethanol), dehidrated in graded ethanol and embedded in paraffin wax at 57°C. Sections 5 μm thick were stained with haematoxylin, observed and photographed under photomicroscope (Jenamed, Carl Zeiss).

RESULTS AND DISCUSSION

Seeds of *J. heldreichii* are fotoblastic same as seeds of *Ramonda* species (Stefanović et al., 1992) and they quickly loose the ability ot germinate. Seeds which we used were fresh providing a nearly 100% germination. Also the method for surface sterilization which was employed provided a high percentage of healthy uncontaminated seedling which was usually higher then 90%.

Among the various explants tested the highest shoot proliferation was obtained in whole plants explants (100%) and cotyledon fragments (80%). Shoots were produced in 50% of leaf explants and 20% of hypocotyl explants. Medium prepared with MS salts was superior in comparison to WPM media.

For long-term maintenance of shoot cultures it was necessary to decrease BA concentration from 5.0 to 0.5-0.1 mg/l. Prolonged exposure to high cytokinin concentration resulted in vitrification and fasciation of cultures and it was therefore considered to be deterimental. Cultures subcultured from shoot induction medium grew well and even multiplied for some time on hormone-free medium, probably due to cytokinin accumulated in plant tissue. Actually, the cytokinin requirement of shoot cultures was small and after the first year spent in *in vitro* conditions it was around 0.2 mg/l BA. The requirement for auxins was also low, IBA at 0.05-0.1 mg/l gave best results in combination with BA.

Characteristic feature of *J. heldreichii* is that apart from axillary buds produced in leaf axils, leaves and leaf pedicels often produce adventitious buds directly on their surface.

Effect of BA on the production of new shoots is presented in Table 1. It is evident that 0.1-1.0 mg/l BA exerts little effect on shoot multiplication via axillary buds which already has a maximum at 0.1 mg/l (5.32). However, BA strongly affects the induction of adventitious buds with maximum at 0.5 mg/l BA on which 67.47% of single isolated leaves produce adventatious buds. At BA concentrations higher than 0.5 mg/l pedicel fail to elongate and cultures appear as small leafy balls. Growth of such cultures is poor since they loose contact with the medium.

Tab. 1. – Effect of 0-1.0 mg/l BA and 0.05 mg/l IBA on formation of axillary and adventitious shoot buds. Subculture duration 30 days

BA (mg/l)	No. of cultures	axillary shoot buds	multipl. index	adventitious buds	
				% of leafs	buds per leaf
0	70	257	3.67 ± 0.2	1.38	1
0.1	59	311	5.27 ± 0.22	25.42	15
0.2	54	268	4.92 ± 0.29	39.06	25
0.5	61	217	3.50 ± 0.25	67.47	42
1.0	55	212	3.85 ± 0.18	67.27	37

J. heldreichii shoot cultures sometimes perish from necrosis associated with gradual browning of the medium. Necrosis appears first in the outer leaf whorld of the rossete. Browning which is usually observed on very soft media seems to be triggered by low pH value of the media (less than 5.8). At pH higher than 5.8 leaves are dark green and media remains translucent.

On media supplemented with auxins apart from rooting of cultures, leaves which were in contact with medium proliferate adventitious buds on the adaxial side and roots on the abaxial side of lamine (Fig. 1 and 2). This morphogenic response of leaves was not observed on hormone-free medium. We thus concluded that in *J. heldreichii* auxin apart from rooting affects also adventitious bud formation. In a treatment in which

single isolated leaves were placed on medium with 0.5 mg/l IBA adventitious buds developed in 13.8% of explants whilst rooting was 100%. Effect of auxins concentration and duration on root length and mean number of roots per explant is presented in Tab. 2.

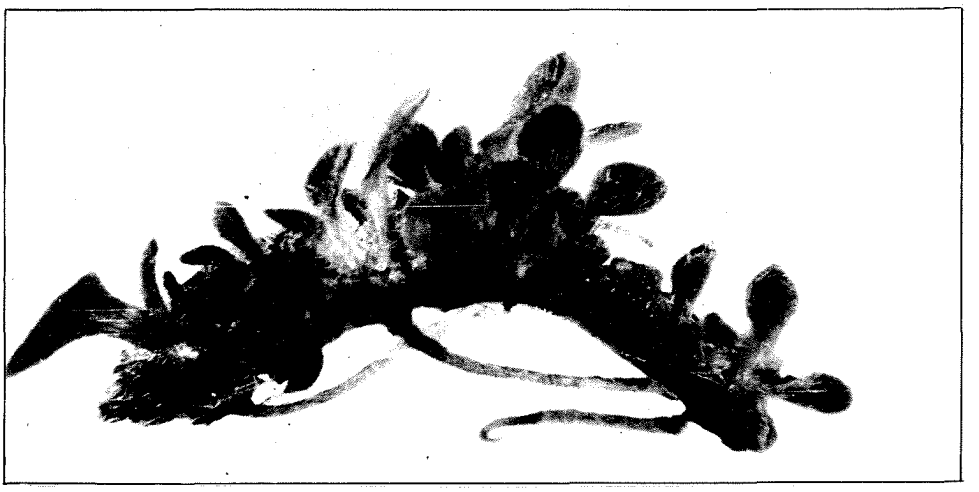

Fig. 1. – Regeneration of adventitious shoot buds on the adaxial and roots on the abaxial side of leafs cultured on MS medium with 0.5 mg/l IBA

Tab. 2. – Effect of the duration of auxin treatment (0.5 mg/l IBA) on root formation and length

auxin treatment days	explants	No of roots	Root length (mm)
9	32	8,0	7.84
12	36	9.11	7.08
16	22	8.5	6.5
19	34	7.67	5.55
30	100	many	2-3

Rooted plants were planted in a mixutre of peat and sand in which they successfully adapted (Fig. 3). However potted plants could not be maintained for more than three months in the glasshouse where they gradually perished from unknown reasons. We assume that the combination of high temeprature and humidity prevailing in our glasshouse was not suitable for this mountain species.

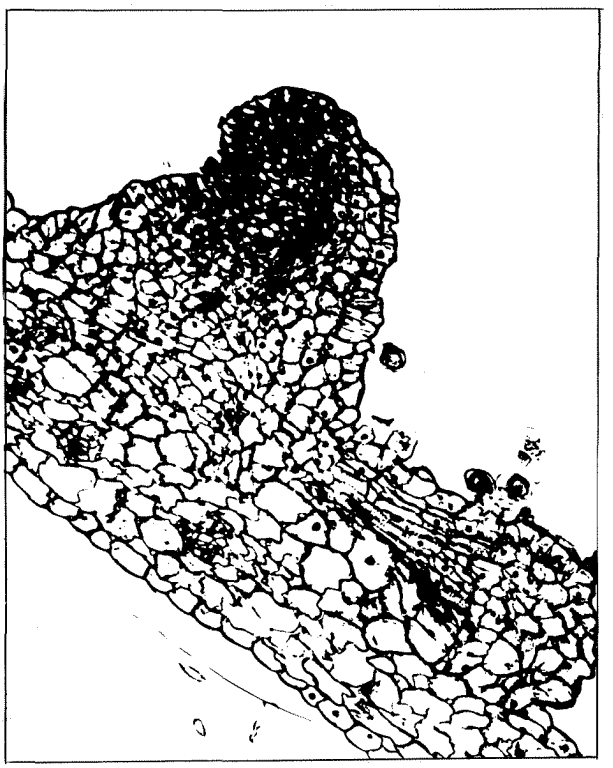

Fig. 2. – Transection through a leaf on medium supplemented with IBA. On the adaxial leaf side an adventitious shoot but is visible in longitudinal section with differentiated vascular elements

Propagation procedure which we employed for *J. heldreichii* was similar to procedures devised for african violet and related species in which induction of shoots was performed by addition of cytokinins to the medium. In some procedures induction medium is supplemented with high cytokinin concentrations (Peck & Cumming, 1974; Start & Cumming, 1976) whilst in others low cytokinin concentration are applied from the beginning of the propagation procedure. However, there are reports (Cooke, 1977; and Radojević et al., 1984) in which shoot multiplication medium apart from cytokinins contains also high concentration of auxins.

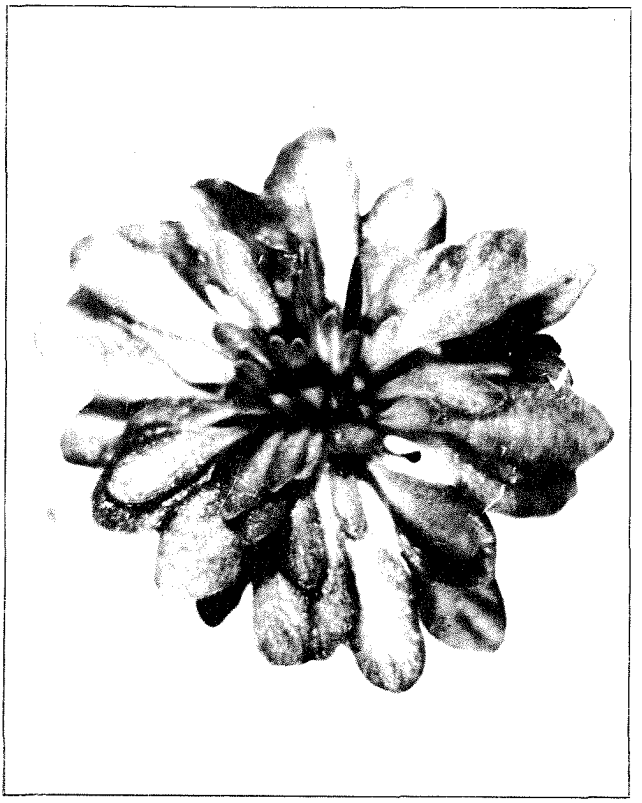

Fig. 3. – Plant propagated *in vitro,* arangement of leaves in the rossete – photographed from above

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Rezime

BRANKA VINTERHALTER, DRAGAN VINTERHALTER, SNEŽANA BUDIMIR

IN VITRO RAZMNOŽAVANJE *JANKEA HELDREICHII* BOISS. *(GESNERIACEAE)*

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Početni eksplantati za uspostavljanje kulture izdanaka *J. heldreichii* Boiss. bili su delovi sterilno isklijalog sejanca (hipokotil, kotiledon, epikotil) ali i cele biljke bez korena. Podloga za indukciju pupoljaka sadržala je MS mineralnu i vitaminsku kombinaciju, 3% saharoze, 0.7% agara a od hormona visoku koncentraciju BAP od 5.0 mg/l i NAA 0.1 mg/l. Optimalna podloga za kulturu rozeta sadrži BAP 0.1-0.5 mg/l i IBA 0.1 mg/l. Na ovoj podlozi pored razvoja bočnih pupoljaka na rubnim listovima rozete indukuju se i adventivni pupoljci. IBA u koncentraciji 0.5 mg/l pored 100% ožiljavanja izdanaka indukuje i pojavu pupoljaka na licu listova pa je neophodno 10-15 dana nakon tretmana sa IBA izdanke prebaciti na podlogu bez hormona. Na taj način sprečava se dalja multiplikacija izdanaka i omogućava izduživanje začetih korenova. 0.5 mg/l IBA indukuje adventivne pupoljke i kod 13.8% izolovanih listova. Adaptacija ožiljenih biljaka u uslovima staklare bila je teška zbog specifičnih uslova u kojima biljka živi u prirodnom staništu.