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Clonal propagation of *Yucca aloifolia* L.

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Abstract

The optimal nutrient media and concentrations of growth regulators for obtaining *Yucca aloifolia* L. plants from seed culture, axillary branching and rooting of microshoots were determined. The common duration of germination of seeds of yucca *in vitro* on the hormone-free Monnier medium was 152 days to achieve 100 % germination. For the first time, modified Quorin and Lepover medium supplemented with 6-benzylaminopurine (1.5 mg l^{-1}) and naphthalene acetic acid (0.06 - 0.1 mg l^{-1}) was used for adventive shoot formation (7 to 8 shoots per explant) in *Y. aloifolia* epicotyl culture. The effects of indole-3-acetic acid, naphthalene acetic acid indole-3-butyric acid, 2.4-dichlorophenoxyacetic acid and its combinations on root proliferation were determined. The best rooting of microshoots of *Y. aloifolia* was found on Murashige and Skoog medium supplemented with 1 mg l^{-1} indole-3-butyric acid and light intensity 800 to 1200 lux. The rate of regenerant survival reached 87.1 % in a soil mixture of garden soil / peat / sand (2:1:1). The obtained results were useded to develop a the scheme of clonal propagation of *Y. aloifolia*.

Key words: growth regulators, *in vitro* propagation, yucca.

Introduction

Aloe Yucca (*Yucca aloifolia* L. – fam. *Agavaceae*) is a small tree with a natural geographic distribution from the North Carolina coast to central Florida and along the Gulf Coastal Plain to Louisiana. It is also occurs in the West Indies and southeastern Mexico (Elias 1989).

In North America, Aloe Yucca has been used as a source of fibres for making ropes, cord etc. (Anisimova et al. 1939). Extracts from *Y. aloifolia* possess oxytoxic and anti-inflammatory properties (Bahuguna et al. 1991). Steroid saponins like smilogenin, tigogenin, neotigogenin, sarsapogenin and gekogenin have been found in leaves of *Y. aloifolia* (Elmunajje et al. 1965; Waclov-Rozkrutowa 1972). Callus obtained from leaves of Aloe Yucca contains 0.82 % sapogenin, including 75 % tigogenin, and significant amounts of gekogenin, gitogenin and cholesterol (Kaneda et al. 1987; Miura et al. 1987). The Aloe Yucca fruit pulp is used in traditional medicine as a laxative and detergent (Kishor, Sati 1990)

Commonly, successful propagation of *Yucca* L. *in vitro* is achieved by induction of proliferation of axillary buds. (Litz, Conover 1978; Kukufchanka, Kromer 1984) At the University of Florida mass propagation of *Yucca* sp. has been achieved through proliferation of adventive buds on Murashige and Skoog (1962; MS) medium containing

naphthaleneacetic acid (NAA) and 6-benzylaminopurine (BAP) (Litz, Conover 1978) The formation of adventive shoots has been observed upon germination of seeds of *Y. shidigera* on MS medium supplemented with 0.3 mg l^{-1} BAP (MacCarthy, Staba 1985). Scientists from the Netherlands developed a method of mass clonal micropropagation of chimerical *Y. elephantipes* Regel., which was also based on the induction of axillary branching by applying MS medium in the presence of 1 mg l^{-1} BAP and 0.1 mg l^{-1} NAA (Pierik, Steegmans 1983). Earlier, in our laboratory we developed procedures of mass propagation of *Y. torreyi* Shafer based on induction of axillary branching on Quorin and Lepover (1977; with 2 mg l^{-1} BAP and 0.4 mg l^{-1} NAA) and MS (with 1 mg l^{-1} BAP and 0.1 mg l^{-1} NAA) nutrient media, which allowed to receive 6 to 14 adventive shoots from one explant (Karpov 2000).

Being the only representative of a genus capable of forming seeds without artificial pollination, *Y. aloifolia* is a potential source for industrial production of steroid saponins. Also, the application of *in vitro* clonal micro-propagation opens new opportunities for duplicating selection forms and hybrids of yucca (Mitrofanova 1997; Butenko 1999; Pierik 1999).

Materials and methods

Juvenile plants obtained *in vitro* in seed culture were used in the present investigation. Isolated seeds were placed on hormone-free Monnier (1968) medium (M). The seed germination was estimated as the ratio of germinated to planted seeds. The frequency of regeneration (R) was measured as a percentage of epicotyls forming adventive shoots.

The efficacy of clonal micropropagation was estimated according to the following formula:

$$K = \frac{N - n}{n};$$

where: N – final number of microshoots;
n – initial number of explants;
K – efficiacy of micropropagation.

Seeds were sterilized in two stages: (i) superficial sterilization of a fruit with 96 % ethanol and (ii) sterilization of the isolated seeds with 70 % ethanol (1 min), followed by rinsing in sterile distilled water. Seeds (10 to 17 seeds per 200 ml flask) were placed on hormone-free M medium.

In the experiments on adventive shoot formation epicotyl culture was used. Microshoots were cultivated on QL medium in two variants with full or a half concentration, supplemented by BAP (0.0; 0.5; 1.0; 1.5; 2.0 and 2.5 mg l^{-1}), 0.04 mg l^{-1} indole-3-acetic acid (IAA), 0.02 - 0.4 mg l^{-1} indole-3-butyric acid (IBA), 0.02 - 0.4 mg l^{-1} naphthalene acetic acid (NAA) and 0.04 mg l^{-1} 2.4-dichlorophenoxyacetic acid (2.4-D) containing 6 % of agar, pH 5.8. Cultures were incubated in a growth cabinet at 22 - 24 °C under a 16-h photoperiod (1000 - 1200 lux).

For microshoot rooting half-strength MS medium was used, which contained 25 mg l^{-1} of iso-inositol, 15 g l^{-1} of sucrose, 55.6 mg l^{-1} $FeSO_4$ $7H_2O$ + 74.6 mg l^{-1} Na_2EDTA, pH 6.5. Auxins were added in the following combinations:

(i) 1 mg l^{-1} IAA; (ii) 1 mg l^{-1} NAA;

(iii) 1 mg l^{-1} IBA; (iv) 2 mg l^{-1} IBA;
(v) 1 mg l^{-1} 2.4-D; (vi) 0.5 mg l^{-1} IAA + 0.5 mg l^{-1} NAA;
(vii) 0.5 mg l^{-1} IAA + 0.5 mg l^{-1} IBA; (viii) 0.5 mg l^{-1} NAA + 0.5 mg l^{-1} IBA;
(ix) 0.9 mg l^{-1} IAA + 0.1 mg l^{-1} 2.4-D; (x) 0.9 mg l^{-1} NAA + 0.1 mg l^{-1} 2.4-D;
(xi) 0.9 mg l^{-1} IBA + 0.1 mg l^{-1} 2.4-D.
MS medium without growth regulators was used as a control.

Results

Germination of the first seeds started on the 9th day of cultivation, and finished on the 152nd day with the 100 %-level of germination. The epicotyls from these seedlings were placed on QL medium containing 0.4 mg l^{-1} of NAA and BAP in various concentrations. Growth of the isolated epicotyls was observed in a range of BAP concentration varying from 0.5 up to 2.5 mg l^{-1}, and axillary branching was observed only in BAP concentration range of from 1.0 to 2.0 mg l^{-1}. The most active axillary branching was observed on QL medium containing 1.5 mg l^{-1} BAP and 0.4 mg l^{-1} NAA. At a decrease of NAA concentration to 0.04 mg l^{-1} and addition of BAP in concentrations of 0.5, 1.0 and 1.5 mg l^{-1}, axillary branching was observed only on medium, containing 1.5 mg l^{-1} of BAP.

Decreasing the concentration of auxin from 0.04 to 0.02 mg l^{-1} and replacing NAA by IBA, IAA or 2.4-D, the most active axillary branching occurred on QL medium containing 1.5 mg l^{-1} BAP and 0.02 mg l^{-1} IBA. The QL medium containing 1.5 mg l^{-1} BAP and 0.02 mg l^{-1} NAA appeared to be ineffective for axillary branching.

When auxins was used in the ratio 1.5 mg l^{-1} BAP to 0.04 mg l^{-1} auxin (NAA, IBA, IAA and 2.4-D), axillary branching was noted only on media, containing NAA or IBA. Thus, the level of efficacy of micropropagation was higher on the medium with NAA (K = 0.8) than on medium with IBA (K = 0.73). However, the frequency of regeneration (R) in the last case was higher (42.3 % against 29.3 % in the variant with NAA).
We also investigated the effect of half diluted QL medium on axillary branching. The pH, concentration of sucrose, iso-inositol and growth regulators remained stable. Axillary branching was seen only on QL medium supplemented with 1.5 mg l^{-1} BAP and 0.04 mg l^{-1} IBA (K = 0.4, R = 40 %). In all other variants (BAP/NAA, BAP/IAA, BAP/2.4-D), axillary branching was not observed.

Using QL medium containing 0.06 mg l^{-1} NAA and 1.5 mg l^{-1} BAP, the number of axillary shoots reached seven (K = 0.83, R = 56.5 %). Replacing NAA by IBA appeared not to be effective (K = 0.38, R = 40 %).

The most active formation of axillary shoots was on QL media supplemented with 1.5 mg l^{-1} BAP + 0.08 mg l^{-1} NAA (max – 8 shoots; K = 0.95, R = 64.2 %) and 1.5 BAP + 0.1 mg l^{-1} NAA (max – 7 shoots; K = 1.04, R = 76 %) (Table 1, Fig. 1).

To determine the optimal medium for rooting of microshoots, the effects of different growth regulators were compared alone and in combinations (Table 2).

Optimal root and shoot growth was observed on MS medium containing 1 mg l^{-1} IBA. Increasing the IBA concentration to 2 mg l^{-1} resulted in callus formation instead of rooting. Light intensity affected rooting of shoots with normal rooting observed in a range from 800 to 1200 lux. An increase of light intensity to 2000 lux resulted in callus formation. The rate of regenerant survival reached 87.1 % in mixture of garden soil / peat / sand at 2:1:1 ratio.

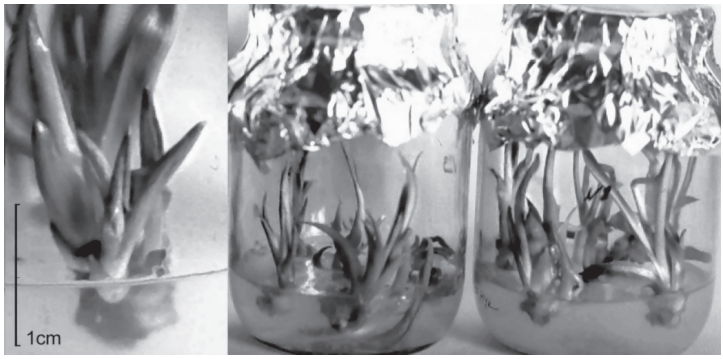

Fig. 1. Axillary branching of *Yucca aloifolia* on QL medium containing 1.5 mg l^{-1} BAP and 0.08 mg l^{-1} NAA.

Table 1. Basic characteristics of axillary shoot-producing cultures of *Yucca aloifolia*. Means ± SE are indicated

Medium and concentrations of growth regulators (mg l^{-1})	Length of maternal shoot (cm)	Characteristics of adventive shoots			K	R (%)
		n_{max}	N ± n	length (cm)		
QL, 1.5 BAP + 0.06 NAA	9.0 ± 3.0	7	3.5 ± 2.3	2.9 ± 2.3	0.83	56.5
QL, 1.5 BAP + 0.08 NAA	7.6 ± 3.2	8	4.0 ± 4.0	3.8 ±3.1	0.95	64.2
QL, 1.5 BAP + 0.1 NAA	6.5 ± 3.5	7	3.5 ± 3.5	4.6 ± 4.0	1.0	76.0

Table 2. Rooting of microshoots of *Yucca aloifolia* and their morphology under the influence of various auxins on the 30th day from the beginning of cultivation. *, abortive roots

Medium and concentrations of growth regulators (mg l^{-1})	Rooting			Callus-forming shoots (%)	Number of chlorotic leaves
	Rooted shoots (%)	Total number of shoots	Number of rooted shoots		
MS	83	12	10*	0	4
MS, 1 IAA	13	32	4	0	2
MS, 1 NAA	0	40	0	70	4
MS, 1 IBA	71	34	24	0	1
MS, 1 2.4-D	0	34	0	100	2
MS, 0.5 IAA + 0.5 NAA	0	22	0	100	2
MS, 0.5 IAA + 0.5 IBA	36	44	16	0	2
MS, 0.5 NAA + 0.5 IBA	0	42	0	43	2
MS, 0.9 IAA + 0.1 2.4-D	0	36	0	39	2
MS, 0.9 NAA + 0.1 2.4-D	13	32	4	33	3
MS, 0.9 IBA + 0.1 2.4-D	0	18	0	100	2

Discussion

The present investigation was conducted in the Nikita Botanical Garden (Yalta), in the Department of Plant Biotechnology and Biochemistry, as a part of the programme on introduction and propagation of yuccas and other arboreal monocotyledonous plants. The main reason complicating the introduction of yuccas is the absence of the natural pollinators – moths of the genera *Tegeticula* and *Parategeticula* (Pellmyr 1999). Therefore, mass seed propagation of these plants are impossible. Vegetative propagation of the most decorative yuccas is also complicated by very low rate of propagation (Novikova 1998).

Y. aloifolia is the sole representative of the genus able to form the seeds in the absence of natural pollinator. Therefore, Aloe Yucca was considered to be the best model object for the numerous experiments on *in vitro* clonal micropropagation of yuccas. Our previous investigations indicated that the easiest way to establish aseptic culture of yucca is to use seed and isolated embryo culture (Novikova et al. 1998, Karpov 2000). In the present experiment, the common duration of *in vitro* seed germination of *Y. aloifolia* on hormone-free Monnier medium (1968) takes 152 days to achieve 100 % germination.

The best formation of axillary shoots of *Y. aloifolia* (max 7 - 8 shoots, K = 0.38 - 1.04, R = 40 - 76 %) in epicotyl culture was observed on QL medium supplemented by 1.5 mg l^{-1} BAP and 0.06 - 0.1 mg l^{-1} NAA. The results are consistent to those obtained earlier in Netherlands for chimerical *Y. elephantipes* Regel., for which the quantity of formed axillary shoots was 7 - 8 per explant (Pierik, Steegmans 1983). As shown by the present investigation, our earlier results (Karpov 2000) and other studies, the best formation of axillary shoots of yucca is usually noted on nutrient media modified with BAP and NAA addition (Litz, Conover 1978; Pierik, Steegmans 1983, Kukufchanka, Kromer 1984). The Quorin and Lepover medium (1977) was used for the first time for axillary shoot-formation in yuccas.

The best rooting of microshoots of *Y. aloifolia* was observed on Murashige and Skoog (1962) medium with 1 mg l^{-1} IBA and light intensity from 800 to 1200 lux.

In accordance the obtained results, optimal clonal propagation of *Y. aloifolia* can be presented as a scheme (Fig. 2).

In conclusion, the results of present study can be used as the basis for clonal propagation of hard-breeding representatives of genus *Yucca* and hybrids that are not able to form the seeds and have a low rate of traditional vegetative propagation.

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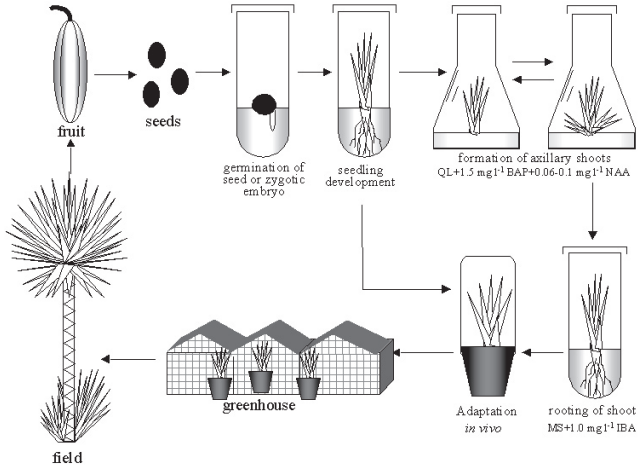

Fig. 2. Scheme of clonal propagation of *Yucca aloifolia* L.

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