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Short communication

Factors affecting in vitro propagation of *Yucca glauca*

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Abstract. A micropropagation system was developed to facilitate the release and subsequent testing of unique pink- or white-flowered selections of *Yucca glauca*. Shoot tip explants from mature plants were cultured on Murashige and Skoog medium supplemented with factorial combinations of α-naphthaleneacetic acid (NAA) (0.0 to 3.2 μM) and 6-benzylaminopurine (BA) (0.0 to 45 μM). Shoots were found to proliferate with increasing concentrations of BA and to produce callus and poorer quality shoots in the presence of NAA and the absence of BA. The response to BA and NAA was similar among 3 genotypes examined. A comparison of BA and N^6-(Δ^2-isopentenyladenine) (2iP) showed that 2iP was not effective in promoting shoot proliferation. Shoot tips rooted in the absence of growth regulators or in the presence of low concentrations of indole-3-butyric acid (IBA). Plantlets were successfully acclimated to greenhouse conditions.

Introduction

Yucca glauca is a species with short woody stems, stiff, narrow, sword-like leaves, a perennial life cycle, and which ranges from Texas to the northern plain regions of North America [1]. It is usually moth-pollinated and takes 5 or more years to flower from seed [1]. Interest in unique flower colour and cold-hardiness in species for use in the urban landscape led to the evaluation of open-pollinated *Yucca glauca* Nutt. collected in South Dakota and hybrids resulting from controlled pollination of these plants. Several superior pink- or white-flowered and potentially cold-hardy plants were selected from 1978 to 1984 in a cooperative project between the US National Arboretum, Washington, DC and the National Plant Germplasm Quarantine Center, Glenn Dale, MD. Traditional propagation by rhizomes, cuttings and offsets yielded few plants for testing and distribution. Successful tissue culture propagation procedures were reported, however, for closely

related plants in the Agavaceae and Liliaceae and in the genus *Yucca* [8, 12, 13]. To facilitate the release of these new *Yucca* genotypes for large-scale nursery production and testing, research was conducted to develop rapid procedures for multiplication in vitro, rooting, and acclimation. Variation between genotypes also was investigated.

Materials and methods

Plant material, culture conditions. statistical analysis

Initial explant material was collected between the months of June and August from vegetative shoots of mature, 6- to 7-year old, field-grown *Y. glauca*. Outer leaves were peeled away beginning at the basal end until a 0.4- to 0.8-cm leafy shoot tip remained. The base of the explant was further trimmed until the total explant was approximately 1 cm in length and 0.5 cm in width. Explants were disinfested for 15 to 20 min in a 1.05% sodium hypochlorite solution and rinsed three times in sterile, distilled water. Explants were placed singly in 25 × 100 mm test tubes containing 10 ml of a standard initiation medium and covered with opaque plastic caps. The standard medium was composed of basal medium (Murashige & Skoog medium [10]), 0.6% Difco Bacto agar, 3 μM 6-benzylaminopurine (BA), and 1 μM α-naphthaleneacetic acid (NAA) to generate stock cultures. The pH of the medium was adjusted to 5.7 with 1 N KOH or 1 N HCl prior to sterilizing in an autoclave at 120 °C and 1.05 kg/cm^2 for 15 minutes.

Cultures were maintained at 25 ± 2 °C under cool-white fluorescent lights at 30 to 55 μmol sec^{-1} m^{-2} on a 16 h light/8 h dark cycle and were subcultured every 4 weeks. Twenty to 30 replicates, each containing a single shoot trimmed to 2 cm in length, were used per treatment for each experiment at each subculture. Tubes were completely randomized. A single genotype was used for all studies except Experiment 5 which evaluated the effect of genotype.

Shoot proliferation investigations were evaluated after three subcultures unless otherwise indicated. Shoot length, root number, percent rooting, and shoot number (large ($\geq$ 1.0 cm), small ($<$ 1.0 cm), and total) were determined. Callus production was rated on a scale of 1 (no visible callus) to 3 (greater than 9 mm^3). Total quality rating (suitability for repeated subculturing or for successful transplanting following rooting) was evaluated on a scale of 1 (poor) to 3 (good).

Statistical analysis of main effects and interactions was made using log-linear models [5]. Traditional analysis of variance was not appropriate

because the model assumptions could not be met, even after transformation of the data. When significant effects were found in experiments with complete factorial designs, response surface regression techniques were used. For experiments with nonfactorial designs, mean separation was done by pair-wise chi-square tests with Type I error adjusted to match that for Duncan's multiple range test [15].

Experiments

Experiment 1. Wounding, BA and NAA. The effects on shoot proliferation of two wounding levels (wounded and non-wounded), four NAA levels (0.0, 0.32, 1.0 and 3.2 μM) and five BA levels (0.0, 1.0, 3.2, 10.0 and 32.0 μM) were tested in a 2 × 4 × 5 factorial arrangement of treatments. Wounding involved removing approximately one-third of the explant by cutting longitudinally through the explant and base. The experiment was evaluated after 4 weeks.

Experiment 2. Time in culture. Stability of shoot production over time (12 weeks) in culture was evaluated using the five non-wounded treatments of Experiment 1 which produced the largest numbers and highest quality shoots. Treatments were: 0.0 μM NAA and 32.0 μM BA; 0.32 μM NAA plus 10.0 or 32.0 μM BA; and 1.0 μM NAA plus 10.0 or 32.0 μM BA. The experiment was evaluated after three 4-week subculture periods.

Experiment 3. Elevated concentrations of BA and NAA. Results of Experiment 1 suggested that optimal BA levels had not been reached. Consequently, five BA levels (9.0, 18.0, 27.0, 36.0, 45.0 μM) and five NAA levels (0.0, 1.0, 1.6, 2.2, 3.2 μM) were tested in a 5 × 5 factorial arrangement of treatments.

Experiment 4. 2iP compared to BA. A comparison of the effects of BA and n^6-Δ^2(isopentenyladenine) (2iP) at 10.0 and 32.0 μM on shoot proliferation and quality was made at two levels of NAA (0.32 and 1.0 μM).

Experiment 5. Genotypes. The original genotype (Genotype 1) plus 2 additional genotypes (Genotypes 2 and 3) were cultured using the five growth regulator treatments of Experiment 2. Genotypes were compared after four 4-week subculture periods.

Experiment 6. Rooting. The effect on rooting of 3.2 and 32 μM indole-3-butyric acid (IBA) and NAA, incorporated into basal medium or applied as

liquid quick dips (20 to 30 sec) prior to placement on basal medium was evaluated after four weeks.

Results and discussion

Experiment 1. Wounding, BA and NAA

Wounding. In general, few differences in proliferation or plant quality resulted from wounding explants. Wounded shoots (w) differed from non-wounded shoots (nw) only in number of large shoots ($\bar{Y}_w = 3.69$, $\bar{Y}_{nw} = 3.65$) and regression analysis showed that the response to growth regulators was the same in wounded and non-wounded treatments. Results contrast with those reported for *Y. elephantipes* [12] in which wounding explants, by splitting in half longitudinally, doubled the number of shoots produced. Possible explanations include differences due to species and/or to degree of wounding. Based on our results, wounding treatments were eliminated from further study.

BA and NAA. NAA had no effect on shoot number, but increasing concentrations of BA produced significant increases in proliferation (Fig. 1). Highest total number of shoots was produced at 32.0 μM BA, while highest number of large shoots was produced at 10.0 and 32.0 μM BA. The difference in response between large and total shoot number was attributed to increased production of small shoots at higher rates of BA (Fig. 1).

Evaluation of shoot quality was made by rating cultures for callus production, and total quality. Low callus production and high total quality were desired to maintain vigorous cultures and decrease the likelihood of aberrant types which may be associated with adventitious shoot production through a callus stage [2, 4, 14]. Callus production and total quality were dependent upon both BA and NAA. The least amount of callus occurred at the lowest levels of BA and NAA (Fig. 1). Unacceptable production (rating ≥ 2.5) was found only at the higher NAA levels in the absence of BA. Highest total quality was found over a range of NAA and BA concentrations (0.32 or 1.0 μM NAA, with 3.2 or 10.0 μM BA) (Fig. 1). All treatments receiving 0.0 μM BA were of unacceptable quality and resulted in roots, few shoots and/or callus.

Experiment 2. Time in culture

Among the five treatments from Experiment 1 selected for 3 successive

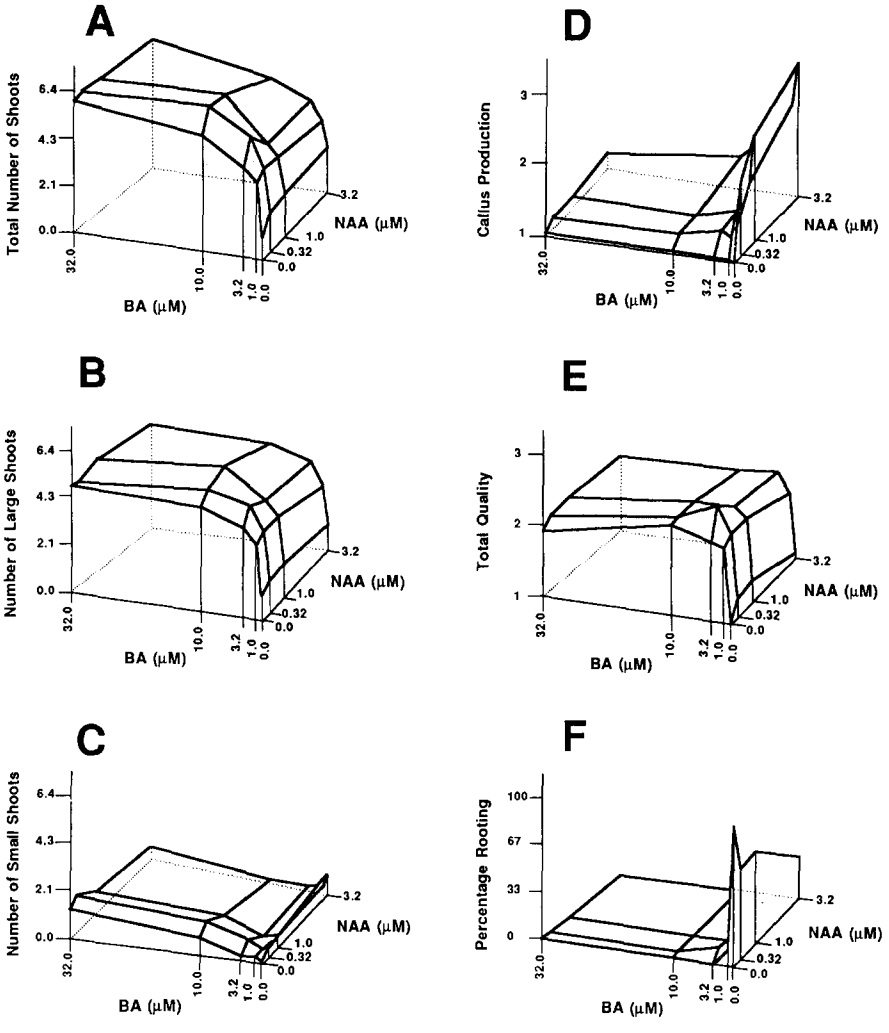

Fig. 1. Influence of BA and NAA on explants of *Yucca glauca*. Data presented are means of non-wounded treatments. Significant effects ($P < 0.05$, statistical analysis by log-linear models) were: (*A*) BA; (*B*) BA, wounding; (*C,D*) BA, NAA, NAA × BA; (*E*) BA, NAA; (*F*) BA, NAA × BA.

subcultures, no differences due to treatment were found for any variables evaluated except shoot length. Treatments with 10.0 μM BA had greater shoot lengths than those with 32.0 μM BA. Highest shoot numbers again were produced at 32.0 μM BA.

A comparison of the results of corresponding treatments at 4 and 12 weeks (Experiment 2 vs. Experiment 1) showed significant differences between experiments for shoot number, callus production and total quality

(data not presented). Compared to results at 4 weeks, total rating and large and total shoot numbers at 12 weeks were slightly higher, while callus production was lower. These observations indicate that increased time in culture may enhance plant quality and proliferation. Consequently, further shoot proliferation experiments were evaluated after a minimum of three 4-week subculture periods.

Experiment 3. Elevated concentrations of BA and NAA

Shoot numbers continued to increase and shoot length continued to decrease with increasing concentrations of BA (Table 1). Highest numbers occurred at 36.0 and 45.0 μM BA. Although NAA had a statistically significant influence on shoot number, shoot length and callus production, trends were not clearly defined. Contrasts with Experiment 1 were attributed to differences in the range of growth regulators tested. There was little varia-

Table 1. Response of *Yucca glauca* explants to elevated levels of BA and NAA.*

Response category	NAA (μM)	BA (μM)					Significant effects ($P < 0.05$)**
		9.0	18.0	27.0	36.0	45.0	
Total shoot number	0.0	5.9	7.0	7.5	6.8	8.1	NAA
	1.1	4.6	6.6	6.4	8.1	7.4	BA
	1.6	5.9	6.7	7.6	8.1	7.9	
	2.2	6.0	5.0	6.1	7.4	7.7	
	3.2	5.4	6.0	5.9	5.8	7.3	
Large shoot number	0.0	5.4	6.6	6.9	6.4	7.4	NAA
	1.1	4.4	6.4	5.9	7.2	6.6	BA
	1.6	5.2	6.3	6.9	7.5	7.4	
	2.2	5.5	4.8	6.0	7.1	7.2	
	3.2	5.1	5.8	5.7	5.6	6.6	
Shoot length (cm)	0.0	3.9	3.2	3.2	2.4	2.8	NAA
	1.1	4.0	3.8	3.2	3.3	2.8	BA
	1.6	3.6	2.7	3.4	3.1	3.1	NAA × BA
	2.2	4.2	4.0	3.6	3.0	2.6	
	3.2	4.3	3.7	3.7	3.0	2.9	
Callus production	0.0	1.3	1.4	1.4	1.4	1.5	NAA
	1.1	1.5	1.4	1.2	1.3	1.4	
	1.6	1.6	1.4	1.3	1.6	1.4	
	2.2	1.1	1.1	1.3	1.3	1.1	
	3.2	1.3	1.5	1.0	1.3	1.3	

*Treatment means are presented. Large shoot number refers to the number of shoots > 1.0 cm. Callus production rated on a scale of 1 (no visible callus) to 3 (> 9 mm^3).
**Statistical analysis by log-linear models.

tion in total quality rating ($\bar{Y}_{min} = 2.5$, $\bar{Y}_{max} = 2.9$, no significant effects) or callus production in the range of growth regulator concentrations evaluated, and all plants had acceptable quality (Table 1).

Shoot proliferation in *Y. glauca* may be increased by raising the concentration of BA to 45.0 μM or higher before a decrease in shoot length interferes with the ability to manipulate shoots; however the effects of elevated shoot proliferation rates on phenotypic stability are unknown. To date all regenerated plants appear normal, but have yet to flower.

Experiment 4. 2iP compared to BA

2iP proved to be much less effective than BA in inducing shoot proliferation. Treatments with 2iP also had lower total quality and higher callus production than those containing BA (Table 2). All treatments containing 2iP produced roots ($\bar{Y} = 54\%$) while those containing BA did not. There were no significant differences in root production among treatments containing 2iP.

Root production in the presence of 2iP has been reported in cultures of the closely related *Y. elephantipes* (shoot cultures) [12] and *Allium cepa* (callus cultures) [6]. One of several explanations for root production and a lack of shoot-promoting activity by 2iP in members of the genus *Yucca* may be that 2iP is being inactivated by a naturally occurring cytokinin oxidase ($(n^6$-isopentenyl) adenosine oxidase) which degrades 2iP but not BA [7]. Such activity has been demonstrated in *Zea mays* [16] and *Phaseolus* [9] and hypothesized in the family Ericaceae [11].

Table 2. Effects of BA and 2iP concentrations on shoot production and rooting in *Yucca glauca.**

Cytokinin (μM)		NAA (μM)	Total shoot number	Cultures rooting (%)
BA	10.0	0.32	5.0 a	0 b
	32.0	0.32	5.4 a	0 b
2iP	10.0	0.32	2.1 b	50 a
	32.0	0.32	2.6 b	53 a
	10.0	1.00	1.8 b	68 a
	32.0	1.00	2.3 b	45 a

* Treatment means within columns followed by the same letter are not significantly different ($P < 0.05$) by pair-wise chi-square tests with Type I error adjusted to match that for Duncan's multiple range test.

Table 3. Comparison of responses of explants of three genotypes of *Yucca glauca* on media containing five combinations of NAA and BA.*

Response category	NAA (μM)	BA (μM)	Genotype			Significant effects**
			1	2	3	
Total shoot number	0.32	10.0	3.0 a	3.7 a	4.6 a	genotype
	1.00	10.0	2.7 a	–	4.9 a	
	0.00	32.0	3.5 a	3.0 a	4.8 a	
	0.32	32.0	3.2 a	3.5 a	4.2 a	
	1.00	32.0	3.5 a	3.5 a	5.8 a	
Callus production	0.32	10.0	1.1 a	1.9 a	1.0 a	genotype
	1.00	10.0	1.1 a	–	1.1 a	
	0.00	32.0	1.2 a	2.2 a	1.2 a	
	0.32	32.0	1.2 a	2.2 a	1.2 a	
	1.00	32.0	1.1 a	1.7 a	1.1 a	
Total quality	0.32	10.0	2.8 a	2.8 a	3.2 a	genotype
	1.00	10.0	2.6 a	–	3.6 a	treatment
	0.00	32.0	2.9 a	2.0 b	3.0 a	genotype
	0.32	32.0	2.7 a	2.1 b	3.2 a	× trt
	1.00	32.0	2.9 a	2.2 b	3.3 a	
Cultures producing adventitious plantlets on leaves (%)	0.32	10.0	0	0	0	genotype
	1.00	10.0	0	–	0	
	0.00	32.0	0	37	0	
	0.32	32.0	0	13	0	
	1.00	32.0	0	13	0	

*Treatment means within a response category for a genotype followed by the same letter are not significantly different ($P < 0.05$), by pair-wise chi-square tests with Type I error adjusted to match that for Duncan's multiple range test.
**Statistical analysis by log-linear models.

Table 4. Rooting response of *Yucca glauca* plantlets to auxins applied as 20-sec liquid dips or incorporated into basal medium.*

Growth regulator		Treatment method	Rooting (%)	Roots/ plantlet	Callus production	Total quality
None		in media	100 a	3.6 a	1.0 c	2.6 a
IBA	3.2 μM	dip	100 a	3.9 a	1.1 c	2.8 a
IBA	32.0 μM	dip	89 ab	3.2 a	1.5 bc	2.3 a
IBA	3.2 μM	in media	95 a	2.7 a	2.1 b	2.1 a
IBA	32.0 μM	in media	28 cd	0.6 b	2.9 a	1.1 b
NAA	3.2 μM	in media	44 bc	0.9 b	2.9 a	1.0 b
NAA	32.0 μM	in media	0 d	0.0 b	3.0 a	1.0 b

* Treatment means within columns followed by the same letter are not significantly different ($P < 0.05$), by pair-wise chi-square tests with Type I error adjusted to match that for Duncan's multiple range test.

Experiment 5. Genotypes

For all measured responses, significant differences were found among the three genotypes evaluated. Total quality was the only response that was significantly affected by media or media/genotype interactions (Table 3). Adventitious shoot production was observed on outer attached leaves of proliferating cultures of Genotype 2. Of seven genotypes which have been placed in culture, Genotype 2 has been the sole selection to produce such adventitious shoots, which are not phenotypically distinguishable from shoots derived from shoot tips. Detached leaves have failed to form adventitious shoots.

Experiment 6. Rooting and acclimatization

Rooting responses observed in Experiment 1 showed that BA was clearly inhibitory to rooting and that the presence of NAA was unnecessary, since 90% rooting occurred on growth-regulator-free media (Fig. 1). Rooting did not occur at BA concentrations greater than or equal to 3.2 μM except for three treatments in which there was only 2 to 3% rooting.

Experiment 6 confirmed that the addition of auxins NAA or IBA as root dips or incorporated in media did not improve rooting over controls (Table 4). The rooting inhibition resulting from NAA was equal to or greater than that from IBA when incorporated into the media. Lower percent rooting, root number, total quality and greater callus production occurred in these treatments. Dips in 3.2 and 32.0 μM IBA and 3.2 μM IBA incoporated into the media did not differ from each other or the control in rooting effectiveness.

Roots, once initiated, grew rapidly and by 4 weeks were often spiraled at the base of the test tube. Rooted plantlets were transplanted into soilless medium and acclimated to ambient (greenhouse) conditions under clear plastic tents with 90% survival after 4 weeks. Further investigations have shown that shoots may be rooted ex vitro, although special attention must be given to monitoring moisture content of potting media to avoid rotting.

Conclusions

Yucca glauca is amenable to in vitro propagation, producing 6 to 8 shoots per subculture. Recent data indicate that long-term (up to 8 weeks), cold/dark storage at 4 °C is feasible, and shows no negative effect on culture performance. Field performance of rooted tissue-cultured plantlets of 7

genotypes is currently being evaluated in four states through the US National Arboretum cooperative evaluation program [3].

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