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EFFECTS OF ABSCISIC ACID ON PHOTOSYNTHESIS, GROWTH AND DEVELOPMENT OF STAGE III *ARONIA ARBUTIFOLIA* (ROSACEAE)

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Additional index words. micropropagule, leaf morphology, woody plant, carbon exchange rates.

Abstract. **Three experiments were conducted to evaluate the effect of ABA on photosynthesis, growth and development of Stage III rooting of *Aronia arbutifolia* (L.) ELL. micropropagules. In the first experiment Stage II micropropagules were transferred onto agar solidified Woody Plant Medium (WPM) supplemented with either 0, 0.1, 1, 10 or 100 mg/L abscisic acid (ABA) in the presence or absence of 1 mg/L indole-3-butyric acid (IBA). In the second experiment, 1 mg/L of IBA and 0, 0.1, 0.5 and 1 mg/L of ABA were used. The final experiment consisted of a 4 x 4 factorial with 0, 2, 4, and 6 mg/L of ABA and 0, 1, 2 and 3 mg/L of IBA. Significant reductions in growth were obtained with increased ABA levels in the first experiment. In the second experiment, negative carbon balances were recorded in Stage III micropropagules under 0, 0.1, 0.5 and 1 mg/L. At lower levels, ABA had no effect on shoot length, leaf area, number of leaves and roots produced. In addition, scanning electron microscopy (SEM) did not show any morphological differences at low ABA levels. In the third experiment, however leaf area and shoot growth were significantly reduced with increased ABA levels independent of the IBA level used. Differences in leaf morphology were observed in treatments with 4 mg/L ABA or greater.**

In vitro produced plantlets desiccate and quickly deteriorate when transferred to an improper acclimatization environment (22, 18). Acclimatization has been defined as the process by which organisms adapt to man-made environments (2). During the acclimatization process, tissue cultured plantlets undergo changes in leaf morphology (5) and physiology (10, 11) which occur over an extended period and confers the plants with a greater potential of survival ex vitro.

Endogenous ABA has been shown to play a role in controlling plant growth and development and alleviating water stress (17). Exogenously applied ABA inhibited the growth rate (6) by inducing stomatal closure (25, 1) or possibly by altering gene expression (4). Other studies have shown that ABA enhanced growth. Hall and McWha (12) found that exogenously applied ABA initially inhibited growth, but after a short lag, increased in leaf and tiller numbers in wheat. Sen et al., (20) observed enhanced shoot morphogenesis in loblolly pine exposed to ABA. Abscisic acid has also mediated water stress by induced changes in stomatal differentiation and leaf cuticle development in aquatic micropropagated heterophyllic angiosperms (14, 9). Similarly, leaves of acclimatized micropropagated plums exhibited greater stomatal density and increased cutin formation (3). Endogenous ABA may play a role in the acclimatization and carbon exchange rates of in vitro regenerated plantlets, but no work has been done to demonstrate this relationship. Under conventional micropropagation protocols, negative and low carbon exchange rates occurred when regenerated plantlets were grown in heterotrophic conditions (10, 21).

The objectives of this study were to characterize the effects of exogenous ABA on photosynthesis, growth and development of Stage III rooted plantlets of *Aronia arbutifolia.*

Materials and Methods

Aronia arbutifolia (L.) Ell. is a woody plant native to Florida with potential as a landscape plant and for use in revegetation efforts (7). The micropropagation protocol used was an adaptation of Kane et al. (13). Stems with lateral buds were cut from actively growing mature plants. Stems were divided into 15 mm length with 2 to 3 lateral buds. The severed explants were rinsed in tap water for 1 hr and surface sterilized by immersion in 50% (v/v) ethanol for 1 min and in 1.05% (v/v) sodium hypochlorite for 12 min, followed by three 5-min rinses in sterile deionized water. Explants were transferred aseptically into 25 x 150 mm culture tubes containing 15 ml of medium consisting of WPM, salts and vitamins (16), 3% (w/v) sucrose, 1 mg/L N^6-benzylaminopurine and solidified with 1.0% (w/v) Sigma Type A®agar. Medium pH was adjusted to 5.5 with 0.1 N KOH before autoclaving at 1.2 Kg cm^{-2} for 20 min at 121°C. All cultures were then grown under a 16-hr photoperiod provided by cool-white fluorescent lamps at

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45 μmol $m^{-2}s^{-1}$ measured at culture levels. Ambient air temperature was maintained at 25 ± 2°C. Cultures, consisting of callus and multiple shoots without roots, were routinely maintained by subdividing and transferring onto fresh media every 5 to 7 weeks.

Stage III rooted plants were generated by cutting 5 week old shoots from in vitro cultures into 10-mm stem segments consisting of 2 or 3 nodes. Fifteen microcuttings were transferred into 473-ml clear polypropylene culture vessels (Better Plastics, Kissimmee, Fla.) containing 100 ml of WPM supplemented with 1 mg/L of indole-3-butyric acid (IBA). Synthetic ABA (90% mixed isomers, Sigma Chemical Co., St. Louis, Mo.) was prepared as a concentrated aqueous stock solution and sterilized by Millipore filtration (pore size: 0.22 μm) before being added to molten (40°C) sterile WPM medium.

Three separate experiments were conducted. The first experiment was a factorial design with four levels of ABA (0, 0.1, 1, 10 and 100 mg/L) and two levels of IBA (0 and 1 mg/L). Culture vessels were randomized in a complete block arrangement with three blocks. This first experiment was undertaken to define the effects of a wide range of ABA levels on growth and development of *A. arbutifolia*. In the second experiment, ABA at each of four levels (0, 0.1, 0.5 and 1.0 mg/L) was combined with 1 mg/L of IBA. The experimental design was a randomized complete block with 5 blocks. The data were analyzed using the General Linear Model (GLM) procedures (19). The third experiment consisted of four levels of IBA (0, 1, 2 and 3 mg/L) factorial arranged with four levels of ABA (0, 2, 4 and 6 mg/L). The data were analyzed using GLM and regression. Total leaf area per plant, shoot growth and total roots per plant were measured at 30 days after transfer to the Stage III media.

First and second fully developed leaves closest to the apex, produced in vitro in experiments 2 and 3, were excised at the end of the experiment and examined using SEM. Leaf samples were fixed in formalin-acetic-alcohol (FAA) for 24 hr at room temperature. Samples were then rinsed and dehydrated through an ethanol series (25 to 100%), critical point dried, mounted on aluminum stubs and sputter coated with gold. Samples were then viewed with an Hitachi SEM-450 under 20 Kv. Photosynthetic rates of plants in experiment 2 were determined at the end

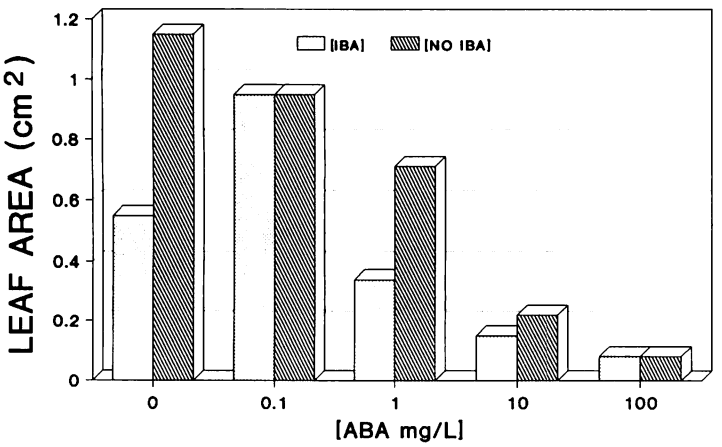

Fig. 1. Influence of 0, 0.1, 1, 10 and 100 mg/L ABA on total leaf area per plant of *Aronia arbutifolia* after 30 days cultured in vitro. Each value represents the mean response of 20 plants.

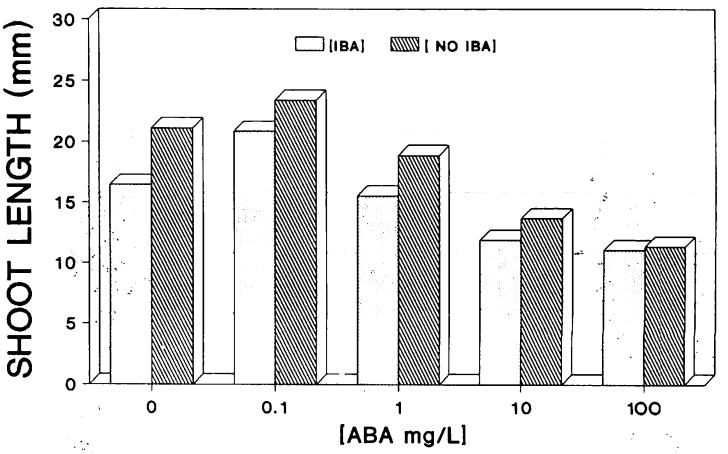

Fig. 2. Influence of 0, 0.1, 1, 10 and 100 mg/L ABA on shoot length of *Aronia arbutifolia* after 30 days cultured in vitro. Each value represents the mean response of 20 plants.

of the experiment with a Hansatech D. W., Clark-type O_2 electrode (Hanastech Limited, Norfolk, England).

Results and Discussion

Experiment 1. Increased ABA levels significantly reduced total leaf area per plant (Fig. 1) and shoot length (Fig. 2) independent of IBA. A greater number of roots developed in the treatments supplemented with 1 mg/L of IBA, yet fewer roots developed in the absence of IBA (Table 1). The level of 0.1 mg/L of ABA slightly enhanced leaf area and shoot growth. Medium supplemented with 1 mg/L ABA slightly reduced growth while ABA levels > 1 mg/L inhibited growth (Fig. 1 and 2). Leaf and shoot growth reduction was more pronounced in plantlets supplemented with 1 mg/L of IBA and 0 and 0.1 mg/L ABA. This reaction was probably caused by an increase in root to shoot ratio of the explants when supplemented with IBA (Table 1). Media supplemented with 10 mg/L ABA or greater was inhibitory to rooting and growth.

Experiment 2. In the second experiment the lower levels (0, 0.1, 0.5 and 1 mg/L) of ABA tested failed to significantly enhance or reduce plant growth (Fig. 3 and 4). Compared to the control, SEM observations of the ABA treated plantlets did not show differences in leaf surface morphology either on the abaxial or adaxial surfaces. Carbon exchange rates were negative regardless of ABA treatment (Fig. 5). Other investigators have obtained negative carbon exchange rates for in vitro plantlets in the absence of ABA (10, 21). No significant differences were observed in root number, yet increased levels of ABA reduced rooting per-

Table 1. Influence of abscisic acid (ABA) on in vitro rooting of *Aronia arbutifolia* plantlets after 30 days.

ABA (mg/L)	Rooting (%)		Number of Roots[z]	
	IBA	NO IBA	IBA	NO IBA
0	33	43	4.2 ± 2.8	2.4 ± 1.3
0.1	70	37	2.9 ± 2.1	2.3 ± 1.5
1	30	65	2.0 ± 1.0	2.2 ± 1.1
10	5	5	1.0	1.0
100	0	0	0	0

[z]Each value represents the mean number of roots per plant ± S.E.

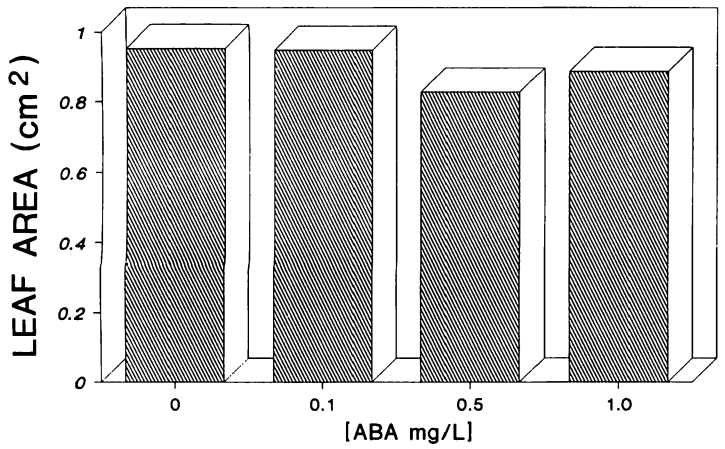

Fig. 3. Influence of 0, 0.1, 0.5 and 1 mg/L ABA on total leaf area per plant of *Aronia arbutifolia* after 30 days cultured in vitro. Each value represents the mean response of 35 plants.

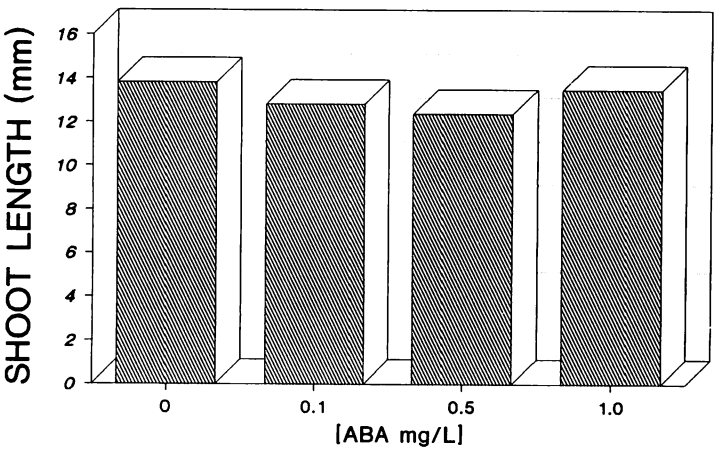

Fig. 4. Influence of 0, 0.1, 0.5 and 1 mg/L ABA on shoot length of *Aronia arbutifolia* after 30 days cultured in vitro. Each value represents the mean response of 45 plants.

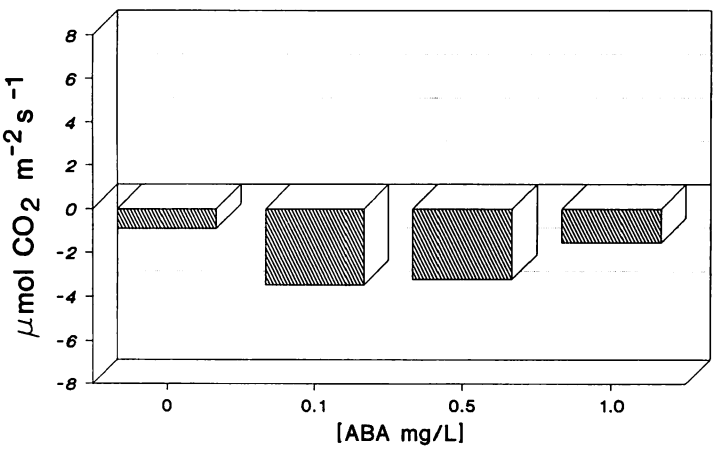

Fig. 5. Influence of 0, 0.1, 0.5 and 1 mg/L ABA on carbon exchange rates of *Aronia arbutifolia* after 30 days cultured in vitro. Each value represents the mean response of 35 plants.

centage (Table 2). In summary, these levels of ABA screened failed to significantly alter in vitro growth and development or carbon exchange rates in this species.

Table 2. Influence of abscisic acid (ABA) on in vitro rooting of *Aronia arbutifolia* plantlets after 30 days.

ABA (mg/L)	Rooting (%)	Number of Roots[z]
	IBA (1 mg/L)	
0	50	7.8±3.7
0.1	42	6.4±4.2
0.5	27	5.5±4.0
1.0	28	8.0±5.3

[z]Each value represents the mean number of roots per plant ± S.E.

Table 3. Influence of abscisic acid (ABA) on in vitro rooting of *Aronia arbutifolia* plantlets after 30 days.

	Rooting (%)				Root Number[z]			
	IBA (mg/L)				IBA (mg/L)			
ABA mg/L	0	1	2	3	0	1	2	3
0	44	90	90	82	1.9±1.1	5.9±3.3	8.2±4.2	6.4±3.5
2	3	0	35	23	1.0±1.0	0	2.5±1.7	3.0±0.9
4	0	0	7	9	0	0	2.0	2.3±1.1
6	3	0	18	21	1.0±1.0	0	1.2±0.4	1.3±0.5

[z]Each value represents the mean number of roots per plant ± S.E.

Experiment 3. In the third experiment, intermediate ranges of ABA concentrations (0, 2, 4 and 6 mg/L) were examined. Percent rooting and root number were inhibited at higher ABA levels (Table 3). A quadratic response was obtained for growth with increased ABA at the various IBA levels tested (Fig. 6 and 7). Plantlets grown in the absence of IBA produce less total leaf area per plant and shoot length per plant. The diminished production of roots observed in the treatment without IBA, probably contributed to a reduction in nutrient uptake from the medium (Table 3) and possibly resulted in lower growth rates. Growth was reduced in treatments with greater than 2 mg/L of ABA (Fig. 6 and 7). Compared to the plants grown in the control medium (2 mg/L IBA and 0 mg/L ABA), the surface morphology of leaves produced during

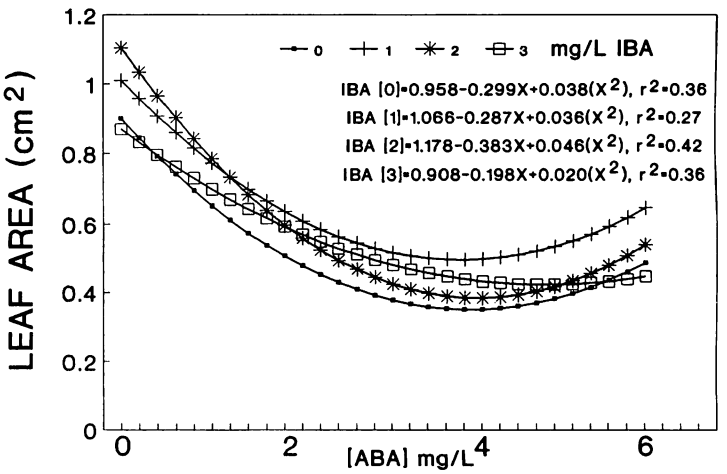

Fig. 6. Influence of 0, 2, 4 and 6 mg/L ABA on total leaf area per plant of *Aronia arbutifolia* after 30 days, as affected by 0, 1, 2 and 3 mg/L IBA. Each value represents the mean response of 25 plants.

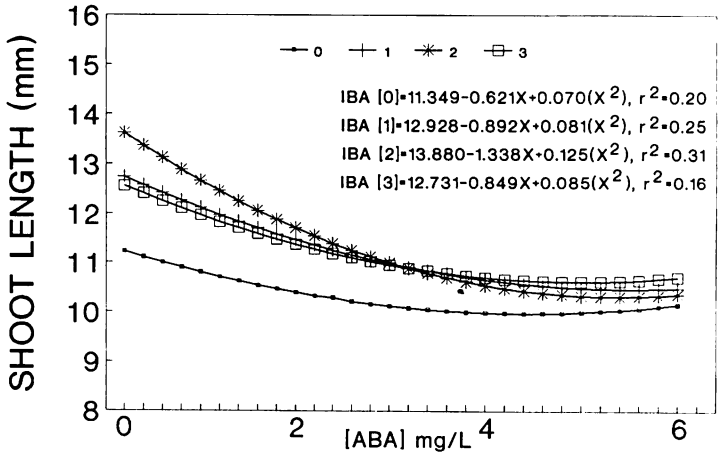

Fig. 7. Influence of 0, 2, 4 and 6 mg/L ABA on shoot length of *Aronia arbutifolia* after 30 days, as affected by 0, 1, 2 and 3 mg/L IBA. Each value represents the mean response of 25 plants.

treatment was altered with increased ABA levels of 4 mg/L or more. In this plant species stomates are confined to the abaxial surface. The abaxial and adaxial leaf surface of the control exhibited smooth epicuticular wax deposition and spherical stomata (Fig. 8A, 8B). The abaxial and adaxial leaf surface of treatments with 4 mg/L and 6 mg/L ABA showed heavy deposition of horizontal rodlets of epicuticular wax on the abaxial and adaxial surface and more elliptical stomata (Fig. 8C-8F). Media supplemented with ABA at 4 mg/L or greater resulted in production of leaves in vitro which morphologically resembled greenhouse produced leaves. Other researchers have shown that these morphological changes occur in in vitro plantlets following transfer to the greenhouse environment (8, 24, 23, 15). The main conclusions which can be drawn from these studies are that ABA applied in vitro can cause development of leaves characteristic of acclimatized plants and that ABA treatments did not affect carbon exchange rates under in vitro conditions. These preliminary results

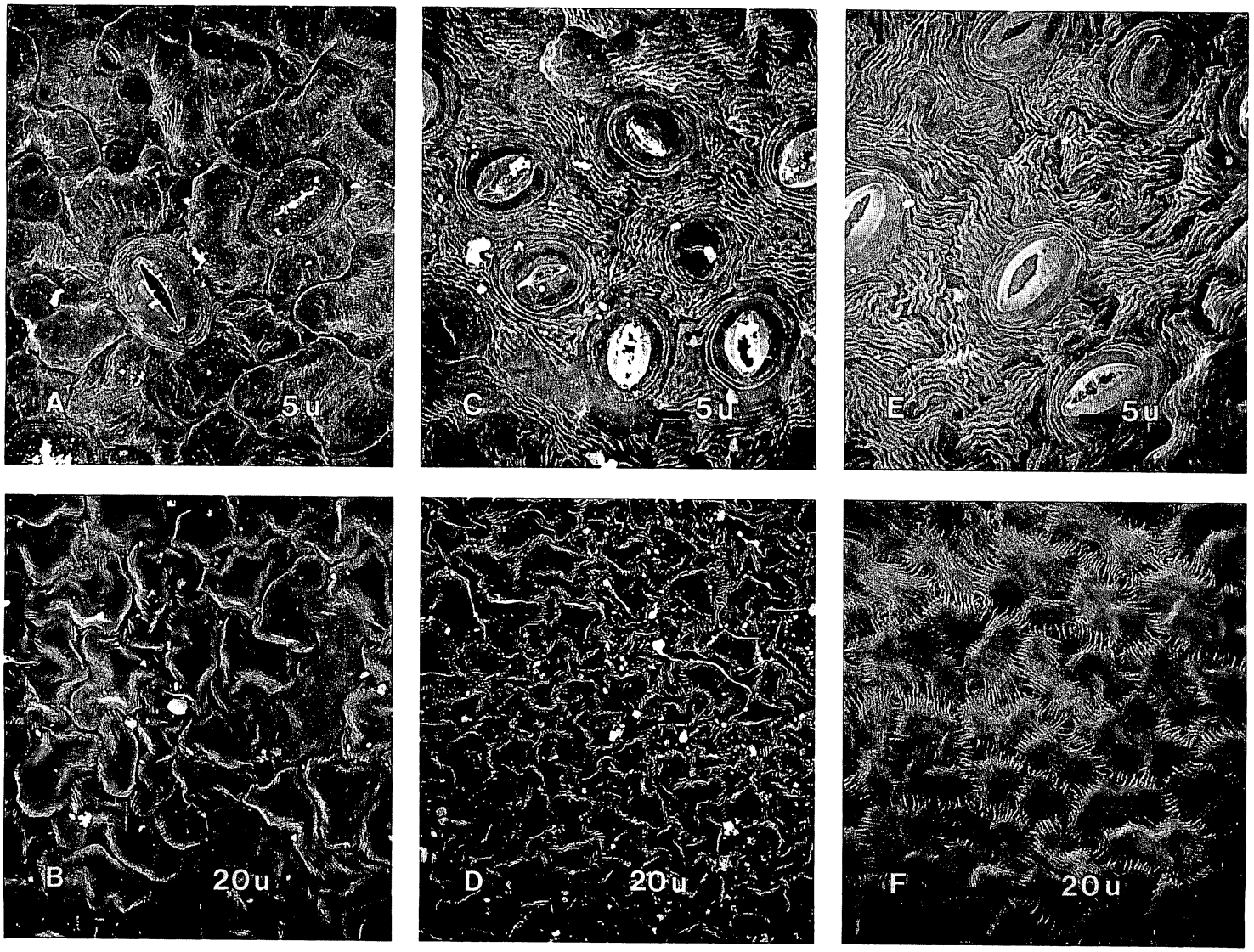

Fig. 8. A. Abaxial and B. adaxial leaf surface of *Aronia arbutifolia*, after cultured in vitro for 30 days (control: no ABA and 2 mg/L IBA); C. abaxial and D. adaxial leaf surface (treatment: 4 mg/L ABA and 2 mg/L IBA); E. abaxial and F. adaxial leaf surface (treatment: 6 mg/L ABA and 2 mg/L IBA).

suggest that ABA treatments may be useful for in vitro preconditioning of Stage III plantlets prior to ex vitro acclimatization. More importantly, this procedure may prove useful for ex vitro establishment of problematic species. Further studies need to address the after-effects of exogenous ABA on growth and development of in vitro produced plantlets under ex vitro conditions.

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EVALUATION OF THE CULTUSAK™ IN VITRO PLANT CULTURE SYSTEM

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Additional index words. aquatic plants, culture vessel, micropropagation, woody plants.

Abstract. **The Falcon CultuSAK™ (CK) is a commercially available heat sealable *in vitro* culture system consisting of an autoclavable 1.25 ml thick gas permeable (non-porous) polyethylene sheet partitioned into five cells each having the approximate volume of a standard 150 x 25 mm glass culture tube. Comparisons were made of the *in vitro* growth of the aquatic-wetland plants: *Cryptocoryne becketii* Thwaites ex Trimen, *C. lucens* de Witt, *Myriophyllum heterophyllum* Michx., *Pontederia cordata* L., *Orontium aquaticum* L.; the woody plants: *Rosa* 'Red Minimo', *R.* 'Royal Ruby', *R.* 'Red Summit', *Aronia arbutifolia* (L.) Pers., *Photinia* X 'Fraseri' Dress; and strawberry *Fragaria* X *ananassa* Duchesne cultured in CKs and standard 150 x 25 mm glass culture tubes. In 73% of the species tested, no significant differences in shoot regeneration rate were observed between cultures maintained in the CKs or culture tubes. Shoot regeneration of *Myriophyllum* was significantly greater in culture tubes. Both *Orontium* and *Aronia* exhibited significantly greater shoot multiplication rates in the CK system. No differences in either dry weight accumulation or rooting were observed in the eleven test species. The potential application of a totally sealed system for *in vitro* production and marketing of aquarium plants is discussed.**

In vitro plant propagation systems require that culture vessels be used which are partially sealed to maintain sterility. Complete culture vessel closure either by tight capping or the use of sealing films can reduce gas exchange and alter water availability which can adversely affect multipli-

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