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The Effect of Micronutrients and GA on the Growth of *Phalaenopsis* Seedlings in vitro

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ABSTRACT

Aseptically-raised young seedlings of a white-flowered hybrid *Phalaenopsis* orchid, with one root and a single leaf were cultured on two media previously used by Hirmen et al. (1989) and by Duan and Yazawa (1995). The latter medium contains Hyponex and no other mineral salts. Each medium was used with or without the following supplemental micronutrients: 10 mg $MnSO_4 \cdot H_20$, 3.0 mg H_3BO_3, 2.0 mg $ZnSO_4 \cdot 7H_20$, 0.75 mg KI, 0.25 mg $Na_2MoO_4 \cdot 2H_2O$, 0.025 mg $CuSO_4 \cdot 5H_2O$, and 0.025 mg $CoCl_2 \cdot 5H_2O$ per liter of medium. Medium containing Hyponex promoted growth (heavier fresh weight and more and longer roots) over the Hinnen et al. medium when evaluated after 12 weeks. The addition of micronutrients to Hyponex and Hinnen et al. medium resulted in a tripling (1868 vs 668 mg) and doubling (240 vs 119 mg) of the fresh weight, respectively. In a separate experiment, supplementing GA_3 at the rate of 1 mg·L^{-1} to the Hyponex medium doubled seedling fresh weight after 16 weeks of subculture, while the addition of GA_{4+7} at the same rate had no effect on seedling growth.

RESUMEN

Plántulas en etapa de una raíz y una hoja de un híbrido de flor blanca de la orquídea *Phalaenopsis* fueron cultivadas en dos medios utilizados previamente por Hinnen et al. (1995) y Duan y Yazawa (1995). Este último medio contiene Hyponex, sin suplemento de otras sales minerales. Cada uno de los medios fue utilizado con o sin la adición de los siguientes micronutrimientos: 10 mg $MnSO_4 \cdot H_2O$, 3.0 mg H_3BO_3, 2.0 mg $ZnSO_4 \cdot 7H_2O$, 0.75 mg KI, 0.25 mg $Na_2MoO_4 \cdot 2H_2O$, 0.025 mg $CuSO_4 \cdot 5H_2O$, y 0.025 mg $CoCl_2 \cdot 5H_2O$ por litro de medio. El medio que contenía Hyponex promovió el crecimiento (mayor peso fresco y raíces en mayor número y longitud) comparado con el medio do Hinnen et al., al evaluarse a las 12 semanas de la siembra. La adición de los micronutrimientos a los medios con Hyponex y de Hinnen et al., favoreció el incremento del peso fresco a 1,868 y 240 mg respectivamente. En un experimento aparte, la adición de 1.0 mg·L de GA_3 al medio con Hyponex, duplicó el peso fresco de la plántula a las 16 semanas del subcultivo, mientras que la adición de GA_{4+7} a la misma concentración, no tuvo efecto.

Additional index words. orchid, growth regulation, mineral nutrition

Orchids seeds are microscopic and each seed has only a multi-celled embryo and seed coat. The seed lacks endosperm or cotyledons to store nutrients which are needed for seed germination and seedling growth. In natural habitats, the germination and further development of orchid seeds require the assistance of certain fungi which convert polysaccharides into more readily available water-soluble sugars (Ernst et al., 1970a). However, since Lewis Knudson germinated seeds and produced seedling plants of *Cattleya mossiae* with an symbiotic method in 1919, this procedure has become the standard method for mass production of orchids from seeds (Ernst at al., 1970a; Goh, 1990). In addition, these findings gave rise to widespread research on nutrients that are required for optimum germination and seedling growth in vitro. Information was generated on the use of mineral salt combinations, carbohydrates, vitamins, plant hormones, and various growth substances on raising orchids from seeds (Ernst, 1970a, b; Stoutomyer and Cooke, 1989).

Knudson's formula C (Knudson, 1946) that has been used for more than five decades ago is still widely employed in orchid production. Hinnon et al. (1989) used polynomial regression models to give the optimal nutrient concentrations for best growth of *Phalaenopsis* seedlings in vitro. Recently, Yanagawa et al. (1995) studied the effect of Hyponex (Hyponex Co., Marysville, Ohio) on in vitro orchid seedling growth to simplify medium preparation. Although the influence of nutrients on seedling growth has been studied intensively, most studies were limited to macronutrients. Less attention has been paid to the effects of micronutrients or growth regulators in the medium on seedling growth in vitro. Among all orchids, *Phalaenopsis* is perhaps the most important and popular commercial orchids being produced around the world (Duan and Yazama, 1995; Wang, 1997). Many hybrids are produced each year and improved methods for accelerating seedling growth in vitro are needed to shorten production time and to reduce the costs.

The objective of this study was to investigate the influence of supplemental micronutrients and GA to two media on the

growth of *Phalaenopsis* seedings in vitro.

MATERIALS AND METHODS

The capsule of a white-flowered *Phalaenopsis* hybrid was obtained from the Ornamental Horticulture Program of the Texas A&M University System Agricultural Research and Extension Center at Weslaco. After washing it with dilute liquid detergent and rinsing with running tap water, the capsule was soaked for 15 min in a 0.6% solution of sodium hypochlorite. The capsule was then dipped in 96% ethanol and flamed once. Seeds were sown on a germination medium containing Hyponox (Yanagawa et al., 1995) which was reported to provide good seedling growth. Seed cultures were kept at 26 ± 2°C in a culture room under a 16 hr photoperiod provided by cool-white fluorescent tubes giving a photosynthetic photon flux of 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Fourteen weeks after sowing, seedlings wore subcultured in the treatments.

Experiment 1. The seedlings used for subculture trearments had a short root and one leaf with an average fresh weight of 18 mg. Treatments consisted of two media previously used by Hinnen et al. (1989) and by Duan and Yazawa (1995). The Hinnen et al. mediurn contains 637 $mg \cdot L^{-1}$ $(NH_4)2SO_4$, 651 $mg \cdot L^{-1}$ KO_3, 1364 $mg \cdot L^{-1}$ KCl, 250 $mg \cdot L^{-1}$ $MgSO_4 \cdot H_2O$, 150 $mg \cdot L^{-1}$ $CaCl_2$, and 150 $mg \cdot L^{-1}$ $NaHPO_4 \cdot H_2O$. The Duan and Yazawa medium has 3.5 $g \cdot L^{-1}$ of Hyponex (6.5N-2.6P-15.8K, Hyponex Co.), i.e., 228, 91, and 553 $mg \cdot L^{-1}$ of N, P, and K, respectively, and 2 $g \cdot L^{-1}$ of peptone. Both media had 20 $g \cdot L^{-1}$ sucrose, 80 $g \cdot L^{-1}$ banana homogenate, 7 $g \cdot L^{-1}$ agar, and 1 $g \cdot L^{-1}$ powdered activated charcoal. Each medium was used with or without supplemental micronutrients. The concentrations of added micronutrients were 10 mg $MnSO_4 \cdot H_2O$, 3.0 mg H_3BO_3, 3.0 mg $ZnSO_4 \cdot 7H_2O$, 0.75 mg KI, 0.25 mg $Na_2MoO_4 \cdot 2H_2O$, 0.025 mg $CuSO_4 \cdot .5H_2O$, and 0.025 mg $CoCl_2 \cdot 5H_2O$ per liter of medium (Gamborg et al. 1968). Each treatment consisted of three 10 cm x 10 cm clear polycarbonate boxes, each containing nine seedlings. The culture was maintained under conditions described above. Plantlets were evaluated following 12 weeks of subculture. Total plant fresh weight, leaf number, and number and length of roots were recorded.

Experiment 2. Seedlings at the same stage as above with a mean fresh weight of 12 mg were placed on a medium with Hyponex only or Hyponex supplemented with 1.0 $mg \cdot L^{-1}$ GA_3 or GA_{4+7}, added after autoclaving, and then cultured as above. Plantlets were evaluated after 16 weeks of subculture. Total plant fresh weight, leaf number, and number and length of roots were recorded.

RESULTS AND DISCUSSION

Experiment 1. There was a significant interaction between medium and micronutrients on seedling fresh weight and leaf number. Adding micronutrients to the Hyponex medium resulted in seedling fresh weight being three fold (1868 mg) those without the micronutrients (688 mg, Table 1). The addition of micronutrients to the Hinnen et al. medium doubled seedling fresh weight. The Hyponex medium in general resulted in seedlings that were much heavier than those on the Hinnen et el. medium. For instance, when additional micronutrients were used, seedlings on the Hyponex medium had nearly eight times the fresh weight as those on the Hinnen et al. medium. Hyponex medium promoted leaf production over that of Hinnen et al., with no beneficial effect from the micronutrients (Table 1). However, plants on the Hinnen et al. medium produced more leaves when micronutrients were added.

Other studies found that the most growth limiting nutrient appeared to be N, because, within a limit, increasing concentrations of both NH_4^+ and NO_3^- promoted growth of seedling plants in vitro (Ernst, 1970; Hinnen et al., 1989). Growth of young *Phalaenopsis* plants in the greenhouse responded to increasing N concentration more drastically than that of P and K (Wang, 1996). In this study, the N in both media was 16.25 $mmol \cdot L^{-1}$, however, the medium containing Hyponex fertilizer resulted in better growth than the Hinnen et al. medium. The N in the Hyponex medium was 17% NH_4^+N and 83% NO_3^-N, whereas its was 44% NH_4^+N and 56% $N0_3^-N$ in the Hinnen medium. Therefore, the best NH_4-N to NO_3^-N ratio reported by Hinnen et al. (1989) did not appear to be the true optimum when another medium is used. However, since seeds were sown on the Hyponex medium, one might argue that the initial adaptation to this medium may have resulted in better seedling growth during the subculture on this same

Table 1. Total fresh weight and leaf number of *Phalaenopsis* seedlings as influenced by in vitro culture medium and supplemental micronutrients.

Medium	Supplemental micronutrients	Fresh wt. mg	Leaf no.
Hyponex	Yes	1868	3.0
	No	688	2.9
Hinnen et al.	Yes	240	2.3
	No	119	1.6
Significance			
Medium (MD)		**	**
Micronutrient (MIC)		**	**
MD X MIC		*	*

* and **, signigicant at $\partial = 0.05$ and 0.01, respectively.

Table 2. Root number and length of *Phalaenopsis* seedlings cultured in vitro as influenced by medium and supplemental micronutrients.

Medium	Supplemental micronutrients	Root no.	Root length
			mm
Hyponex	Yes	4.0	24.7
	No	3.3	19.9
Hinnen et al.	Yes	2.1	13.7
	No	0.7	5.7
Significance			
Medium (MD)		**	**
Micronutrient (MIC)		**	**
MD X MIC		NS	NS

* and **, significant at $\partial = 0.01$ and non-significant, respectively.

Table 3. Growth of Phalaenopsis orchid seedlings in vitro as influenced by GA_3 or GA_{4+7} at a rate of 1 mg•L^{-1} after 16 weeks of culture on a Hyponex medium.

Treatment	Fresh wt.	Leaf no.	Root length
	mg		mm
Control	300 b[z]	2.5 b	14.7 b
GA_3	613 a	3.1 ab	21.7 a
GA_{4+7}	327 b	3.5 a	15.6 b

medium, as opposed to subculturing on the Hinnen et al. medium.

There was no interaction between medium and micronutrients on root number or length. Greater root number was obtained when cultured on the Hyponex medium. Root length of plants on the Hyponex medium was 230% of those on the Hinnen et al. medium (Table 2). Supplemental micronutrients promoted root growth by increasing both root number and root extension, regardless which medium was used.

Experiment 2. The addition of GA_3 more than doubled seedling fresh weight and increased root length by 50% (Table 2). Since leaf number was unaffected by GA_3, the increase in fresh weight was due to the larger leaves and longer roots. GA_{4+7} had no beneficial effect on fresh weight or root length. However, GA_{4+7} resulted in a greater leaf number than the unamended Hyponex medium. As a result, plants cultured in medium amended with GA_{4+7} had much smaller leaves than plants in the other media. Ganesh et al. (1996) reported that amending culture medium with BA + GA_3 promoted shoot multiplication and growth of *Vanilla*. When measured at five months following transplanting in sphagnum moss and growing in a greenhouse, plants raised on the unamended medium and medium amended with GA_3 or GA_{4+7} had leaf spread of 11.9 ± 1.5, 14.6 ± 1.7, and 5.0 ± 1.9cm, respectively.

From the results of this study, it is clear that choosing the right medium and using the proper concentrations of micronutrients are important to obtaining fast growth of *Phalaenopsis* seedlings. These large seedlings may be transferred out of flasks at an earlier time and/or maintain a growth advantage over the slow-growing smaller seedlings (Konow, 1998). The difference between growth rates and delayed transfer time of slow-growing seedlings in an inferior medium can result in an additional year of greenhouse growing of the smaller plants before flowering, thereby increasing the production costs.

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