rate of increase in callus weight diminished. Therefore, 10% to 15% CW is recommended for spinach medium. Increasing the CW level to 20% gave only a slight increase, particularly with 'High Pack'.

Shoot regeneration. In the absence of CW, calli derived from 'High Pack' regenerated at a frequency significantly higher than that exhibited by 'Baker'. This relationship also held true in the presence of CW. The percentage of calli, which regenerated shoots from 'High Pack' on CW-free medium, was not significantly different from the percentage of regeneration from 'Baker' on CW-enriched medium. A significant interaction of genotype and coconut water was also observed in a study with wheat (*Triticum aestivum* L. em. thell) callus (Mathias and Simpson, 1986). They found that shoot development was promoted by 10% (v/v) CW in some lines but was inhibited in others. In our study, however, shoot formation in both genotypes of spinach was promoted by CW addition, but the degree of increase in regeneration frequency was cultivar dependent.

The percentage of calli that regenerated shoots increased with concentration of CW up to 15% (Fig. 1B). With concentrations >15%, however, shoot regeneration was inhibited in both cultivars tested.

Growth of regenerated shoots of both cultivars was significantly improved as a result of CW enrichment (data not shown). Shoot length of cultivars was unaffected by CW.

Regeneration was substantially expedited with CW enrichment. In the absence of CW, up to 12 weeks of culture was required before shoot formation became apparent. With CW, however, the time required was reduced to 4 to 5 weeks. There was no definite pattern of the onset of regeneration in relation to increasing concentration of CW.

Limiting CW addition to the regeneration stage. Augmenting only the regeneration medium with CW was sufficient to provide a significant improvement in shoot regeneration of spinach. Calli induced on a CW-free medium exhibited a significant increase in regenerative capacity upon transfer to CW-enriched regeneration medium. Regenerative capacity of calli induced on a CW-free medium (33%) and callus induced on a CW-enriched medium (41%) were similar when these calli were transferred onto a CW-enriched regeneration medium. In contrast, calli grown and induced to differentiate on a CW-free medium exhibited significantly less shoot regeneration (15%).

Although CW addition to callus induction medium is not required to achieve high regenerative capacity, when callus multiplication or rapid callus growth is desired, callus induction and continuous subculture of callus on CW-enriched medium are recommended. Regenerating calli should be maintained on CW-enriched regeneration medium if a shoot multiplication phase is used to increase the number of the regenerated shoots. A multiplication step consists of transferring the regenerating calli onto fresh regeneration medium in GA-7 Magenta ves-

sels (Magenta, Chicago) that provide ample space for shoot multiplication.

Stimulation of callus growth and shoot regeneration by CW in two cultivars of spinach has been shown in this study. Enrichment of the culture medium with CW during the regeneration phase of the system would provide the same effect as enriching the media at the callus induction and shoot regeneration phases. The increase in shoot regeneration capacity of callus, the expedited callus proliferation and the shoot differentiation associated with CW addition would maximize potential use of this tissue culture system as a method for the rapid propagation of selected spinach plants.

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Microwave Sterilization of Plant Tissue Culture Media

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Abstract. Nutrient medium can be sterilized using a household-type microwave oven. The required microwave treatment time was influenced by the oven's microwave power intensity (70 to 700 W), vessel type, volume of medium employed, and the presence of energy sink water reservoirs (ESWR). Growth rates of strawberry (*Fragaria vesca* L.) shootlets, lemon [*Citrus limon* (L.) Burm. f.] fruit halves, or carrot (*Daucus carota* L.) callus cultured on either microwaved or autoclaved media were similar. Microwaving and autoclaving appeared to reduce GA₃ activity compared with medium containing filter sterilized GA₃. Chemical name used: gibberellic acid (GA₃).

Autoclaving is the method of choice to sterilize paraphernalia and nutrient media for in vitro culture. The most common alternative method of sterilizing media is filtration through a 0.2-μm sterile filter; however, it is expensive, time consuming, and usually unsuitable for handling large volumes. Gas sterilization, using ethylene oxide, is the most common alternative to autoclaving for sterilization of utensils. Again, this method of sterilization is expensive, time consuming, and involves the use of a highly toxic chem-

ical. Nutrient medium should be autoclaved at 121°C at 105 kPa for 15 min to achieve sterilization (Burger, 1988; Perkins, 1969). However, large volumes of medium have been found difficult to sterilize by autoclaving due to the inherent insulating factors associated with media and containers (Burger, 1988; Perkins, 1969). For example, to sterilize 1500 ml of medium, 35 min of preheating may be required to reach 121°C to begin the subsequent 15-min sterilization period (Burger, 1988).

Domestic household-type microwave ovens have been used to sterilize items including plastic tissue culture vessels (Latimer and Matsen, 1977; Sanborn et al., 1982), agar nutrient media (Nelson, 1990; Wood and Lundergan, 1981), and dental tools (Rohrer and Bulard, 1985). Wood and Lundergan (1981) reported that microwaving of tubes containing agar medium for 2 to 3 min was sufficient to achieve sterility. Unfortunately, no information was given on the microwave dosage used, the number of tubes sterilized simultaneously, or the occurrence of undesirable medium boiling during sterilization. In our initial studies, use of a microwave oven to sterilize nutrient medium caused

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Table 1. Incidence of boiling over and of sterilization during microwave heating of agar nutrient medium in 25-ml aliquots in ten 25 × 150-mm culture tubes with and without energy-sink water reservoirs (ESWR).

Treatments	Tubes (%)	
	Boiled over	Sterile ^a
<i>700 W, - ESWR</i>		
Duration (min)		
1	0.0 a	0.0 a
3	0.0 a	0.0 a
5	100 b	0.0 a
10	100 b	100 b
<i>700 W, + ESWR</i>		
1	0.0	0.0 a
3	0.0	0.0 a
5	0.0	60.0 b
10	0.0	100 b
<i>10 min incubation + ESWR</i>		
Microwave setting (W)		
70 (low)	0.0	20.0 a
210 (defrost)	0.0	40.0 ab
210 (medium/low)	0.0	50.0 ab
350 (medium)	0.0	70.0 bc
490 (high/medium)	0.0	100 c
700 (high)	0.0	100 c
<i>700 W</i>		
Duration 5 min	ESWR no. Vol (ml)	
	1 450	100 a 40.0 a
	1 750	80.0 a 10.0 a
	1 900	40.0 b 40.0 a
	2 250	0.0 b 20.0 a
	2 450	0.0 b 10.0 a
	2 900	0.0 b 0.0 a
<i>700 W</i>		
Duration 7.5 min	1 450	100 a 60.0 a
	1 750	30.0 b 70.0 a
	1 900	40.0 b 100 a
	2 250	20.0 b 100 a
	2 450	30.0 b 70.0 a
	2 900	0.0 b 90.0 a
<i>700 W</i>		
Duration 10 min	1 450	100 a 20.0 a
	1 750	30.0 b 70.0 b
	1 900	80.0 a 100 b
	2 250	20.0 b 100 b
	2 250	30.0 b 100 b
	2 900	0.0 b 100 b
<i>350 W, + ESWR</i>		
Duration (min)		
10	0.0 a	70.0 a
12	0.0 a	90.0 a
15	90.0 b	100 a
20	100 b	100 a

^aTreatment percentages within columns within groups indicated by headings separated by Fisher's exact test ($P < 0.5$). Three replications/treatments conducted. Data presented from a single replication.

boiling and ejection of closures. Further, no medium sterilization was achieved when many tubes containing agar medium were microwaved simultaneously or if 100 to 1000 ml of liquid medium in a single container was microwaved for 2 to 3 min. Therefore, we initiated a study to determine the factors necessary to sterilize agar and liquid media using a microwave oven.

The basal nutrient medium (BM) contained the following constituents, in mg·liter⁻¹: KNO₃, 100; H₃BO₃, 2.5; CaCl₂·2H₂O, 150; CuSO₄·5H₂O, 1; FeSO₄·7H₂O, 3; MgCl₂·6H₂O, 97.8;

MgSO₄·7H₂O, 100; MnSO₄·H₂O, 2.5; KI, 0.5; KH₂PO₄, 5; Na₂MoO₄·2H₂O, 0.25; ZnSO₄·7H₂O, 2; thiamine·HCl, 0.5; i-inositol, 100; sucrose, 30,000; and agar (Flow Laboratories, McLean, Va.), 8000. The pH was adjusted to 5.7 ± 0.1 with 0.1 N HCl or NaOH before the addition of agar. Agar medium was melted and dispensed in 25-ml aliquots into 25 × 150 mm borosilicate glass culture tubes or in 50-ml aliquots into 90 × 150 mm polycarbonate containers that were capped with transparent Bellco (Vineland, N.J.) polypropylene Kaputs or screw caps, respectively. Media were autoclaved for 15

min at 105 kPa and 121°C, and tubes were then slanted at a 45° angle while cooling. Liquid medium was dispensed in 100-, 250-, 500-, and 1000-ml aliquots into 1-liter pyrex bottles and capped with orange polypropylene screw caps. Tubes were microwaved upright in polypropylene culture trays.

A Sharp Carousel II microwave oven, (Model R-5E80; Sharp Electronics Corp., Mahwah, N.J.), having inside dimensions of 390 mm diameter × 252 mm height and turntable revolving at 6 rpm was used throughout this study. This oven produced 700 W at a frequency of 2450 MHz. The microwave could be adjusted for several power settings including: 100% (high), 70% (medium/high), 50% (medium), 30% (medium/low), 30% (defrost), and 10% (low), corresponding to 700, 490, 350, 210, 210, and 70 W, respectively. Unless otherwise indicated, energy sink water reservoirs (ESWR) were included in the microwave oven chamber during incubation when culture tubes and polycarbonate containers were sterilized. ESWR consisted of two 1-liter pyrex borosilicate glass bottles loosely capped with blue polypropylene screw caps and each containing 900 ml distilled water. Temperatures of liquid or agar medium and water in the ESWR, when used, were recorded before and after each microwave incubation treatment, as was the time when media started boiling during microwave treatments.

The influence of the inclusion of ESWR in the microwave oven, to aid in the sterilization of agar BM in culture tubes, was investigated. First, culture tubes containing agar BM, ± ESWR, were subjected to a 700-W microwave intensity for 1, 3, 5, or 10 min. Then, the ability of intensities of 70, 210, 350, 490, and 700 W to sterilize 10 culture tubes containing agar medium was examined during 10 min of exposure with ESWR. Next, the influence of ESWR volumes on the sterilization of agar medium in culture tubes was determined. Reservoirs tested consisted of either one or two 1-liter bottles containing 250, 450, 750, or 900 ml distilled water. Ten tubes containing agar medium were treated with the above ESWR for 5, 7.5, or 10 min. To investigate the ability of low microwave power settings to sterilize medium, 350-W (i.e., 50% power setting) treatments for 10, 12, 15, or 20 min were used in the presence of ESWR to sterilize 10 tubes containing agar medium. A varying number of containers, 5, 10, or 30 culture tubes or 6, 9, 10, 13, 15, or 18 polycarbonate containers, with and without ESWR, were given 210-, 350-, 490-, and 700-W microwave treatments for 2 to 20 min.

To determine a suitable method of sterilizing liquid BM with a microwave, 100, 250, 500, 1000, 2000, and 3000 ml liquid BM were incubated at 5, 10, 15, 20, 25, 30, or 50 min at 700 W. No ESWR were used.

Polypropylene Swinnex disk filter holders, 25 mm diameter (Millipore Corp., Bedford, Mass.) fitted with 25-mm diameter polyvinylidene difluoride membrane filters with 0.2-μm pore size, were sealed in polyethylene freezer bags (ziplock) and treated

Table 2. Influence of number of 95 × 100 mm polycarbonate containers on time required to achieve sterilization using various microwave power settings.

Containers (no.)	Microwave		ESWR	Tubes (%)	
	Duration (min)	Power (w)		Boiled over	Sterile ^a
6	5 ^y	700	—	0.0	16.6 a
6	10	700	—	0.0	100 b
9	10	700	—	0.0	100
10	10	700	+	0.0	50.0
13	15 ^y	700	—	0.0	100
15	15	700	—	0.0	100
18	20 ^y	490	—	0.0	16.6 a
18	20	700	—	0.0	83.3 b

^aTreatment percentages within columns within groups indicated by headings separated by Fisher's exact test ($P < 0.5$). Three replications/treatments conducted. Data presented from a single replication.

^yTime required to achieve boiling of medium.

Table 3. Influence of time of exposure to a microwave intensity of 700 W on the incidence of sterilization of liquid medium (no ESWR used).

No. bottles in oven	Microwave time (min)	Vol (ml)		Boiling time (min) ^a	Sterile (%) ^y
		Per bottle	Lost		
1	5	100	10	1.5	100 a
1	10	250	18	3.0	100 a
1	10	500	15	4.0	100 a
1	10	1000	17	4.0	0.0 a
1	15	1000	30	4.0	100 a
1	20	1000	50	4.0	100 a
2	10	1000	0	8.0	0.0 a
2	15	1000	15	8.0	0.0 a
2	20	1000	25	8.0	0.0 a
2	25	1000	27	8.0	50.0 b
2	30	1000	50	8.0	100 b
3	30	1000	---	12.0	0.0 a
3	50	1000	70	12.0	100 b

^aTime recorded for medium to achieve boiling.

^yTreatment percentages within columns within groups indicated by headings separated by Fisher's exact test ($P < 0.5$). Three replications/treatment conducted and totaled to determine percent sterility.

with 700 W for 5, 10, or 15 min with a single ESWR. In some cases, filters were wetted with distilled water before the microwave treatments. After the filters were treated, they were used to add triplicate 1-ml filtered water samples to autoclaved agar BM in tubes to determine the success of the microwave treatments.

Following microwave treatments, agar or liquid BM in either tubes, containers, or bottles was incubated for 4 to 8 weeks and examined daily for contamination to determine the sterilization success of the microwave treatments. Most often, contamination was detected first as the occurrence of white bacterial growth on the surface or within the medium. Also, 1-ml aliquots from microwaved liquid BM were tested on Potato Dextrose Agar (PDA) and Nutrient Broth (NB) (Sigma, St. Louis.) for occurrence of microorganisms.

'Alexandria' strawberry shootlets were isolated from sterile germinated seedlings and grown on agar BM containing 1-naphthaleneacetic acid (NAA) at 0.1 mg·liter⁻¹ and *N*-(phenylmethyl)-1*H*-purin-6-amine (BA) at 1.0 mg·liter⁻¹. Embryogenic callus of 'Danver's half long' carrot was initiated from petiole segments cultured on BM containing 2,4-dichlorophenoxyacetic acid at 0.1 mg·liter⁻¹. For testing the influences of the microwave method of media sterilization on growth of cultured plants, 1-cm³ pieces of

strawberry shootlets or carrot callus were planted on the surface of autoclaved or microwaved BM with NAA and BA or BM only, respectively. Fruits (35-mm diameter) of 'Eureka' lemon were obtained from 6-year-old trees grown at the Univ. of California, Riverside. Fruits were surface sterilized in a 2.6% sodium hypochlorite solution (containing two drops of Tween-20 emulsifier per 100 ml solution) for 30 min and then rinsed three times with sterile, distilled water. The extreme stylar and stem ends were then severed. Fruits were bisected equatorially, and halves were planted on the surface of BM that contained GA₃ at 10.0 mg·liter⁻¹. Fruits were tested on BM sterilized by: 1) microwaving with GA₃ included, 2) autoclaving with GA₃ included, 3) microwaving with filter-sterilized GA₃ added aseptically later, and 4) autoclaving with filter-sterilized GA₃ added aseptically later.

For each species, 10 to 20 cultures were used. All cultures were incubated in a temperature-controlled environmental chamber at 26 ± 1°C under a 16-h daily exposure to 2.2 W·m⁻² using cool-white fluorescent lamps. Cultures were allowed to incubate for 4 weeks and then examined for contamination and fresh weight increases. All experiments were repeated at least three times. Where applicable, data were analyzed by Fisher's exact test.

Inclusion of ESWR prevented boiling of

media and Kaput ejection from tubes during microwave treatments even at the full microwave setting, i.e., 700 W (Table 1). One- to 3-min microwave treatments with or without ESWR were insufficient to sterilize media. Five- and 10-min microwave treatments without ESWR led to medium boil over and cap ejection due to pressure buildup. Boil over resulted in medium loss and was often a pathway for future contamination. In such cases, caps were retrieved and the tubes re-capped upon completion of microwave treatments. Five minutes of microwaving was insufficient to obtain medium sterility; in contrast, 10 min of microwaving without ESWR resulted in sterilization of all tubes, although all exhibited medium boiled over and most exhibited cap ejection. When the ESWR was included in the oven during the microwave treatments, 10 min was optimum for medium sterilization without any cap ejection or medium boil over. The medium had reached 97°C at the end of this treatment. In contrast, 5-min treatments with ESWR resulted in only 60% of the tubes being sterile with a final medium of 88°C. Similarly, Keller et al. (1988) found that all contaminants were eliminated when 1.5 liter seawater culture medium was microwaved for 10 min. The final temperature of the seawater culture medium was 84°C.

A close relationship occurred between microwave dosage administered for 10 min and the success of sterilization of medium (Table 1). Dosages of 490 and 700 W resulted in complete medium sterilization. On decreasing the dosage intensity settings to 350, 210, or 70 W, proportionally fewer tubes were sterilized.

Varying the volume of the ESWR greatly influenced medium sterilization and boil over (Table 1). Whatever the volume of the ESWR, 5 min of microwave exposure was inadequate to obtain complete sterilization of all 10 culture tubes tested. Low boil over and high sterilization percentages were obtained from 7.5- and 10-min microwave treatments using two reservoirs each containing 250 or 900 ml water (Table 1). Even 10 min with only one ESWR containing 450 ml was insufficient to obtain sterilization due to the severe medium boil over coupled with cap ejection, which resulted in subsequent contamination. Using 50% power intensity, i.e., 350 W, with the ESWR, 15 min was necessary to sterilize agar medium in tubes (Table 1).

Variable rates of sterilization of 95 × 100 mm polycarbonate containers were due to incubation times that varied, the power intensity used, or whether ESWR were included (Table 2).

The geometry of the culture vessel needs to be considered. Microwave treatment of medium in petri dishes, other shallow vessels, or tubes <25 mm diameter resulted in excessive medium boil over with medium loss frequently >75% of the original volume. Microwaving with such vessels, even with ESWR, was impractical. Narrow tubes promote boil over because the medium meniscus rises more readily in such vessels than

in wide-aperture vessels, i.e., 95 × 150 mm containers. We also found that white or colored Kaput closures were not as suitable as translucent or clear types because they were more prone to melting or ejection from tubes during microwaving.

Liquid medium was sterilized effectively by microwaving without ESWR (Table 3). Longer durations of microwaving were required to sterilize larger volumes of solution. While only 5 min was necessary to sterilize 100 ml of medium, 50 min was required to sterilize 3000 ml divided into three 1-liter bottles (Table 3).

Basal medium, PDA, and NB were equally effective in determining contaminants. The time required for the medium to reach its boiling point was recorded in this experiment (data not shown). Apparently, temperatures in liquid media varied greatly; the surface of the medium boiled while medium at the bottom of the vessel was 20°C cooler.

Filters, whether prewetted or not, could be effectively sterilized by microwaving for 5 to 15 min.

Growth of cultured lemon fruits, strawberry organogenic shootlets, or carrot asexual embryogenic callus on microwave-treated media did not differ from growth on autoclaved media. Similar observations, i.e., that microwaving had no adverse effect on media sterilization, have been made by others (Keller et al., 1988; Wood and Lundergan, 1981). However, microwaving appears to partly break down GA₃ since fresh weights of cultured lemon halves on autoclaved and microwaved media containing GA₃ (35.5 ± 0.9 and 34.1 ± 1.4 g, respectively) were similar but were lower than those of cultures grown on autoclaved or microwaved media containing filtered GA₃ (44.4 ± 1.6 and 45.3 ± 2.2 g, respectively).

Using 700 W of power, liquid medium volumes containing 3% sucrose required the following sterilization times, (ml, min): 100, 5; 250, 10; 500, 10; 1000, 15; 2000, 30; and 3000, 50. Fifteen 95 × 100 mm polycarbonate containers, each containing 50 ml agar medium, also could be sterilized using 700 W for 15 min. Ten culture tubes, 25 × 150 mm, containing 25 ml of agar medium, could be sterilized 15 min using 350 W or 10 min using 700 W when two 1-liter ESWR, each containing 900 ml of distilled water, were included in the microwave. Syringe-type filter holders with 25-mm-diameter filters, could be successfully sterilized by microwaving at 700 W for 5 min with an ESWR included.

Microwaving nutrient media, culture vessels, and filters, especially plastics, can be a great advantage in the plant tissue culture. Microwave ovens are considerably less expensive, generate less heat during sterilization, require less energy to operate, are easier to obtain and install, and require less operation time than traditional steam autoclaves. Also, some undesirable features are associated with autoclaved medium, such as pH changes, formation of precipitates due to the interaction of inorganic constituents and other media components, and occurrence of trace

metal contamination resulting from steam impurities. These alterations in media are prevented in microwave sterilization (Keller et al., 1988).

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Monitoring the Potassium Status of Greenhouse Tomatoes Using Quick Petiole Sap Tests

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Abstract. 'Celebrity' tomatoes (*Lycopersicon esculentum* Mill.) were grown in peat-perlite under greenhouse conditions in two experiments with five levels of K (25, 50, 100, 200, and 300 mg-liter⁻¹) in irrigation waters. In both experiments, aqueous petiole sap K concentrations (milligrams K per milliliter) were monitored weekly with colorimetric paper test strips. Variation in petiole sap K concentrations among plants was low among three-plant replicates (CVs of 9% to 11%). Petiole sap K concentrations varied considerably from week to week, but remained relatively constant over time at levels determined by external-feed K concentrations. Petiole sap K levels and marketable-fruit yield increased quadratically with increasing external K concentrations. Maximum yields were produced with 190 to 200 mg K/liter in external-feed solutions. Maximum marketable yields of ≈2.75 kg/plant occurred with ≈5.9 mg K/ml in petiole sap. Quick petiole sap K tests appear to be suitable for assessing the K status of greenhouse tomatoes.

Adequate K availability is crucial to optimum production and quality of tomatoes. Deficiencies of K are associated with a number of economically significant physiological disorders of tomato fruit, including greenback, blotchy ripening, and graywall (Forster, 1973; Picha and Hall, 1981). Excessive K fertilization can depress uptake of Ca and Mg, lead to other serious disorders such as blossom-end rot (BER) (Raleigh and Chacka, 1944), and increase total rhizosphere salts to potentially yield-limiting levels. Environ-

mental concerns over the use of excessive amounts of fertilizers are intensifying and are likely to lead to increased regulation of fertilization practices in the future.

Methodology for quick, grower-conducted tissue tests for nitrate in plant sap is showing promise for use in efficient and environmentally sensitive management of the N status of tomatoes (Coltman, 1988). The ability to simultaneously determine both the N and K status of a crop with quick tests would facilitate greater acceptance and use of quick testing techniques while encouraging growers to reduce excess applications of fertilizer. A preliminary study suggested that the K status of greenhouse cucumbers could be assessed using quick petiole-sap tests (Adams, 1982). The purpose of our experiments was to evaluate the feasibility of using rapid petiole-sap analysis for guiding K fertilization management of greenhouse tomatoes.

Two experiments were conducted in a greenhouse at the Univ. of Hawaii, Hono-

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