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Abstract

Turmeric (*Curcuma longa* L.) plantlets were cultured in liquid medium with 6% sucrose. Microrhizome development was observed in the presence of methyl jasmonate (MeJa) (0, 5 and 16 µM) and benzyladenine (BA) (0, 0.32 and 1µM). Leaf, root, rhizome growth, and sugar use were measured weekly for 6 weeks in small vessels (180 ml) and four times in 23 weeks in larger vessels (2.5 L). MeJa reduced leaf and root biomass and rhizome biomass to a lesser degree. BA had a positive effect on biomass accumulation. Microrhizomes mass increased at a linear rate for 6 weeks in culture while roots and leaves accumulated biomass exponentially. Sugar use correlated nearly directly to whole plant dry weight (DW) in the short and long-term experiments. Microrhizomes became a larger fraction of whole plant DW as plantlets aged. After 6 weeks, about 1.8 g of microrhizome DW per liter of media had been produced (in both time courses), and after 23 weeks, about 26 g of microrhizome DW per liter of media had been produced. Secondary rhizomes were first observed at 14 weeks and most plants had them by 23 weeks. A method for rhizome production in a long-term culture system was described. The relationship with sugar use will be useful in the eventual development of a model for sugar use and secondary metabolite production.

Abbreviations: Benzyladenine – BA; Methyl Jasmonate – MeJa; Dry Weight – DW; Fresh Weight – FW; Plant Growth Regulator – PGR; Relative Dry Weight (DW/FW) – RDW

Introduction

Turmeric (*Curcuma longa* L.), an herb that has been used in traditional Indian medicine for centuries, has gained reinvigorated interest due to its significant medicinal potential. Turmeric is used as a dye, spice, and industrial starch in commercial applications. A yellow textile dye is made from the characteristic yellow-orange curcuminoids found in the rhizomes. Turmeric is a component of curry powder and is popular in the countries of southern and eastern Asia. Ayurvedic medical systems have different uses for both fresh and dried preparations with dried powders being used to treat distinctly different ailments from pastes or plant juices (Kapoor, 1990; Parrotta, 2001).

Roughly 80% of the world's supply of commercial turmeric is produced in India (about 635,950 tons/year) (Ravindran, 2007). Turmeric plants are sterile triploids and must be maintained by repeated vegetative propagation of rhizomes. The divisions are field planted and allowed to grow for one or two years depending on size and quality of propagules (Wardini and Prakoso, 1999). Replanting subterranean divisions in tropical soils spreads fungal and bacterial diseases. Rhizomes are slow to multiply, and yields range from 15-20 tons per hectare (Balachandran et al., 1990).

Turmeric has been clonally propagated in vitro through shoot (Nadgauda et al., 1978) and rhizome callus induction (Yasuda et al., 1988). More recently, microrhizomes were found to develop in vitro in liquid medium with increased sucrose levels correlated with rhizome size. Larger microrhizomes were capable of survival in the field without any discreet acclimatization procedure (Shirgurkar et al., 2001; Salvi et al., 2002). Microrhizomes were also found to produce secondary metabolites with antioxidant activity equaling or surpassing commercial dried powdered rhizome preparations of field-grown plants (Cousins et al., 2007).

There are many reports on methods to induce microrhizome development. Most researchers have used liquid MS medium modified with benzyladenine (BA), a common synthetic cytokinin. Other plant growth regulators, increased sucrose concentration, and modified environmental conditions have also been employed in attempts to promote microrhizome development. Raghu (1997) produced microrhizomes on liquid MS medium supplemented with 1.33 μM BA, 0.54 μM 1-napthylacetic acid (NAA), 1.95 μM ancymidol, and 10% sucrose. Sanghamitra and Nayak (2000) produced microrhizomes on liquid MS medium with 13.32 μM BA, 6% sucrose and a 4 h photoperiod. Shirgurkar et al. (2001) found that 4.4 μM BA allowed microrhizome development while high levels (35.2 μM) of BA could completely inhibit microrhizome formation in turmeric. Intermediate BA levels also caused decreased microrhizome development. Sunitibala et al. (2001) grew rhizomes on liquid MS medium supplemented with 0.54 μM NAA, 4.65 μM kinetin, and 8% sucrose. In our laboratory, in liquid MS medium modified with 6% sucrose and 1 μM BA, increased vessel size led to increased turmeric plant size (though larger vessels of agar did not increase plant size), and rhizomes functioned as storage organs (Adelberg and Cousins 2006). In this medium, 3.2 μM ancymidol was found to reduce fresh weight by about 10% without increasing rhizome mass. We also tested 2%, 4%, 6%, and 8% sucrose media and found that solute partitioning to rhizomes was greatest in 6% sucrose media (Adelberg and Cousins, 2007).

Jasmonic acid increased the size and numbers of storage organs formed in bulb forming species in vitro in sugar containing medium (Norjiri et al., 1992; Ravinkar, et al., 1993; Santos and Salema, 2000; Kim et al., 2003). Jasmonic acid production is often induced in plants as a result of pathogen presence, wounding, or insect attack; its concentration increased in differentiated leaf tissue and cell suspension cultures when exposed to insect attack and fungal

cell wall preparations (Blechert et al., 1995). Many reports state that jasmonate can induce secondary metabolite production (Gaisser and Heide, 1996; Gundlach et al., 1992; Zabetakis et al., 1999). MeJa has been shown to alter expression of phenylalanine ammonia lyase (Sharan et al., 1998) in the shikimic acid pathway (Schmidt and Amrhein, 1995) – a pathway responsible for production of many phenolic secondary metabolites.

Tissue biomass must be evaluated in conjunction with metabolite concentrations since total metabolite production in plant systems is based on biomass as well as concentration. This current study will further characterize the growth of turmeric plants in vitro by observing the effects of plant growth regulators (BA and MeJA) on the development of plant organs over time in large and small vessels. Correlations between sugar use and whole plant and microrhizome growth and development will be drawn. Analysis of secondary metabolite activities from this, and similar studies, is currently being conducted.

Materials and Methods

Plant material

In vitro stock plants. Turmeric accession L35-1 was obtained from the University of Arizona Southwest Center for Natural Products and Commercialization. Stage I was prepared by dissecting the quiescent shoot tips from rhizomes, immersing them in full-strength commercial bleach (Clorox™, 5.25% sodium hypochlorite) for 30 seconds, and plating them on PGR free MS media. Stage II cultures were maintained for at least 6 months by sub-culture every six weeks in liquid modified MS medium (Murashige and Skoog, 1962) that included: 170 mg NaH_2PO_4, 100 mg myo-inositol, 0.25 mg nicotinic acid, 0.25 mg pyridoxine hydrochloride, 0.05 mg thiamine hydrochloride, 60 g sucrose per liter, and 1 µM BA. Medium pH was adjusted to

5.7 before being dispensed. Explants were placed in 180 ml glass jars containing 30 ml of liquid tissue culture medium and cultured on an orbital shaker (100 rpm) with 25 to 35 μmol s^{-1} m^{-2} photosynthetically active radiation provided by cool white fluorescent tubes with 16 h d^{-1} photoperiod maintained at 24 ± 2 °C. Buds were conditioned to lower BA levels (0, 0.32, or 1 μM) for one six-week cycle prior to commencement of PGR factorial time course experiment to minimize BA carryover effects.

PGR factorial in time course experiments

Short-term time course. BA concentrations (0, 0.32, and 1 μM) and MeJa concentrations (0, 5, and 16 μM) were arranged in 9 treatment factor combinations (3 x 3). Shoot and root systems were removed, and two buds conditioned to the experimental BA concentration were placed in each jar containing 33 ml of medium on a shaker (as above). Four predetermined jars from each treatment factor combination were harvested destructively each week for six weeks. Residual sugar concentration in expended liquid media was determined with a refractometer. Plants were dissected and leaves, rhizomes, and roots were grouped together prior to FW and DW determination. Plant dry weight (DW) was recorded after drying the contents of each vessel individually in paper envelopes at 70 ºC for 48 h.

Long-term, fed-batch time course. Large rectangular vessels (approximately 2.5 L), (Liquid Lab Vessels, Southern Sun Inc., Hodges, SC; Adelberg and Simpson, 2004), were agitated on a thin-film rocker system (Figure 1) (Adelberg and Cousins, 2006) that produced a 1 cm pitch every 15 minutes (with a 1 rpm cam). Each vessel contained 200 ml of medium of the same formulation and treatment combinations as used in the short-term time course. Plants were conditioned to BA concentrations (0, 0.32, or 1 μM) prior to the experiment start. Vessels were

inoculated with plant material from one six-week old jar (13-17 plantlets trimmed free of roots) grown at appropriate BA concentration. Two vessels at each of the 9 BA and MeJa treatment-factor combinations were harvested on three dates (6, 14, and 23 weeks) over a 23 week time course. The harvest method was the same as that used for the short-term time course. According to our fed-batch process, media was added to each remaining vessel following the harvest date to return the media volume to approximately 200 ml and sucrose concentration to approximately 6%. Following 6 weeks in culture, two vessels from each treatment condition were harvested as above. Approximately 75 of the 200 ml of medium remained. This residual medium was 3% sucrose compared to the initial 6% sucrose. Therefore, 125 ml of 8% sucrose medium was added to the unharvested vessels to replenish the medium to approximately 200 ml of 6% sucrose medium. After 14 weeks, 90 ml of 1.2% sucrose medium remained in each vessel. As a result, 110 ml of 10% sucrose medium was added to replenish the medium to 200 ml with 6% sucrose. The final group of vessels was harvested at 23 weeks.

Field-grown plants. Plantlets grown on 1 µM BA media in vitro were acclimatized in moist soilless mix (Fafard 3B, Fafard Inc., Anderson, SC) in 1204 plant cell packs under intermittent mist (6 s of mist every 6 minutes during day) for 3 weeks. Subsequently, they were hand-watered in a conventional greenhouse for another 3 weeks. Acclimatized plants were then transferred to a natural soil bed in a covered, heated field structure (Specialty Crops Greenhouse, Fort Valley, GA). Flood irrigation was provided as necessary, and plants were grown from March to December in 2005 (one growing season).

Experimental design and analysis

The experiment was a completely randomized design for the 9 PGR treatment conditions (3 levels of BA and 3 levels of MeJa arranged as a 3 x 3 factorial) with vessels being the unit of replication (4 replicates of small jars in the short-term time course and 2 replicates of large vessels in the long-term time course). One set of vessels was harvested on each of 6 dates for jars and 4 harvest dates for large vessels. The data were analyzed separately for short-term (small vessels) and long-term (large vessels) studies. For the long-term time course, PGR data was pooled as determined to be appropriate by ANOVA with $\alpha = 0.05$. Short-term time course fits were separated using the log likelihood ratio test with $\alpha = 0.01$. Best fits of selected response variables are presented. Statistical analysis was performed using JMP 3.2.6 (SAS Inst., Cary, NC, USA) and SAS Version 9.1 (SAS Inst., Cary, NC, USA), and graphics were created with Statistica Version 7.1 (Statsoft Inc., Tulsa, OK, USA).

Results

Short-term time course in small jars for six weeks

Growth of leaf, root and rhizome biomass (FW and DW) are shown for the 9 combinations of growth regulators over the six weekly observations (Table 1). There was a definite dichotomy between linear increases in mass of rhizome and exponential increases for growth of leaves and roots.

Plantlets accumulated biomass most rapidly in the absence of MeJa in our standard medium containing 1 µM BA. Plantlet growth was negatively impacted by the presence of MeJa (Figure 2a). Plant DW accumulation was slowed by 16 µM MeJa as was rhizome growth (FW and DW accumulation). Leaf FW (Figure 2b) and DW growth over time were negatively impacted by MeJa with both 5 and 16 µM levels yielding reduced leaf mass. Root FW (Figure

2c) and DW displayed similar results with decreased root growth at 16 µM MeJa. Increases in MeJa concentration lowered leaf, root, and plant mass in 1µM BA (Figure 3).

In the lower BA concentration (0.32 µM), plant, root and leaf FW accumulation were negatively effected by 16 µM MeJa while plant DW and rhizome biomass (FW or DW) were unaffected by MeJa. MeJa effects on root and leaf growth were more varied than we observed in 1 µM BA. In the absence of BA, MeJa had no detectible effect on plantlet growth – leaves, roots and rhizomes (FW or DW). Only shoot FW was negatively affected by 16 µM MeJa.

Lowering BA concentrations to 0 µM or 0.32 µM did not promote rhizome development. Furthermore, shoot, root and whole plant growth were not enhanced either. The effects of lowered BA concentrations were only detectable in the absence of MeJa, and plants grown in media with 1µM BA grew best as measured by total FW, rhizome FW and rhizome DW.

Microrhizome FW and DW increased at a linear rate over the short-term time course (Figure 4). RDW (DW/FW) of microrhizomes was approximately 0.10 in small jars. Leaves and roots had lower RDWs than rhizomes (data not shown). MeJa caused an increase in rhizome harvest index (rhizome DW/total DW) after 6 weeks of growth – 45% (0 µM MeJa), 51% (5 µM MeJa), 57% (16 µM MeJa) for DW. BA had no effect on this factor.

Long-term fed-batch time course in large vessels for twenty-three weeks

MeJa and BA concentrations did not influence growth (whole plant FW and DW) in the long-term fed-batch time course in larger vessels with media supplementation (data not shown). Whole plant FW and DW increased over the entire 23 weeks (Figure 5a), but the rate of FW gain declined slightly around 15 weeks. The rate of rhizome DW accumulation increased over 23 weeks (Figure 5b) as did the RDW of the rhizome tissue (Figure 5c). In the long-term time

course, the RDW was approximately 0.10 during the first 6 weeks (similar to jars) but increased to 0.12 and 0.16 by weeks 14 and 23, respectively. Harvest indexes increased over the course of the experiment from 38% at 6 weeks to 47% at 14 weeks and 60% at 23 weeks.

At 14 weeks, secondary rhizomes became evident upon peeling back the leaves and roots covering the rhizomes. These new structures radiated from primary rhizomes in a few of the specimens (Figure 6a). By week 23, leaf blades and roots were visibly atrophied and rhizome enlargement was evident (Figure 6b). When roots and leaves were pulled away, most rhizomes were swollen with secondary rhizomes present (Figure 6c).

By week 23, about 26 g of rhizome DW had been produced per liter of media (based on 435 ml per vessel). The 2.5 L vessels were fed 33 g sucrose, and over 32 g of that sucrose was consumed by the plant material. Over 23 weeks, 11.3 g of rhizome DW and 19 g of total DW were accumulated per vessel. Therefore, about 59% of the sugar mass was incorporated as DW. Rhizome RDWs were 0.16 at 23 weeks, and 0.63 g rhizome DW was produced per plant. When vessels were opened, a pleasant aromatic aroma was observed. Also, rhizomes displayed the yellow color characteristic of curcuminoid phenolics when they were cut.

Relationship between sucrose and biomass

There was a nearly direct relationship (Figure 7) between sugar use and DW. Calculations showed that a 1 g increase in tissue DW required approximately 1.8 g of sugar from the medium. Therefore, approximately 59% of the sucrose gram for gram was incorporated into the plantlet as DW with the remainder being released as CO_2 and water or lost as a result of other plant processes such as phenolic exudation or cell sloughing. In vitro plant cells generally exhibit a conversion efficiency of approximately 50% meaning that half of the organic carbon taken up

from the media is converted to cell DW in bioreactor systems (Curtis, 1999). As a result, we may conclude that the turmeric plantlets in liquid MS medium were of similar efficiency. For each 2 g of sugar used, 0.73 g of plant DW were produced, consisting of 0.37 g rhizome DW, or 0.18 g rhizome DW per plant. After 6 weeks, tissue from jars was of similar quality and size to that generated in large vessels over the same time period (Figure 8).

Field grown system

After 42 weeks of growth in soil, field grown rhizomes from clone L35-1 had an RDW of 0.24. Field rhizomes were 30 to 60 g FW per plant. These rhizomes had deep yellow-orange coloration and released aromatic odors when cut. Field grown plants had greater rhizome mass than linear rates of microrhizome mass increase in jars would predict if extrapolated from 6 to 42 weeks. In the long-term time course, microrhizomes had a constant rate of DW accumulation (Figure 5c) and appeared more like field grown tissue at the final harvest. In vitro microrhizomes at 23 weeks were still growing much slower than field plants. Sucrose concentrations observed at weeks 6, 14 and 23 were 3%, 1.2% and 0.5% respectively. We believe that the plants spent a great deal of time at suboptimal sucrose concentrations. This caused some portion of the difference in growth rates between the field produced and in vitro derived tissues. An optimized fed-batch system would more closely approximate the growth rates seen in field grown turmeric plants.

Discussion and conclusions

Short-term time course in small jars for six weeks

The growth of vegetative tissues typically has a lag phase, followed by an exponential rate of increase until limitation in resources forces a senescent phase. The leaves and roots in the short-term time course showed this characteristic behavior that did not extend to a point of growth limitation by week 6. MeJa delayed the onset of the exponential growth phase with leaves and roots. However rhizomes did not enter the exponential phase of growth during the short-term time course.

BA is an important constituent of the media. This experiment indicated that turmeric had inadequate endogenous cytokinin for optimum rhizome growth and disproves our earlier belief that the 1µM BA concentration in our micropropagation medium was too high for rhizome development.

Long-term fed-batch time course in large vessels for twenty-three weeks

Long-term growth was made possible through fed-batch media supplementation over the time course and enabled formation of functional rhizomes with secondary branching. Also, over time, larger percentages of the total plant biomass was contained in the active storage organ – the rhizome. Despite the addition of fresh medium leaves and roots went into atrophy and began a senescent phase of their growth cycle (Figure 6). Rhizome dry matter accumulation increased in at the expense of leaf and root growth as rhizomes became competitive sinks. The finding that turmeric plants have higher RDW in the rhizome than in the other organs (data not shown) indicates active accumulation of carbohydrates in storage organs and is consistent with prior observations of turmeric microrhizomes in vitro (Adelberg and Cousins, 2006; 2007). The growth rates of in vitro rhizomes were still lower than growth rates in field culture.

The relation of sugar use to DW for the short-term time course were similar to line fits for the long-term time course. A refractometer can be useful to determine when nutrient supplements are needed in a system where sugar is the limitation to growth. Similar factors may describe relationships between secondary metabolite production and DW. Sucrose concentration, as a monitor of biomass prior to harvest, would than characterize, a repeatable and standardized culture method.

Conclusions

The method for long-term culture of turmeric plants described here permit longer culture cycles and produced plantlets with similar in vitro derived microrhizomes shown to contain high levels of antioxidant activity (Cousins et al., 2007). The fed-batch culture system demonstrated in 2.5 liter vessels allowed for large biomass accumulation in the rhizome at time of extraction and analysis. The quality and concentration of secondary metabolites need be addressed in a system that produces large quantities of biomass with a high harvest index.

Alternatively, larger microrhizomes may be used as propagation stock to make robust field plants to rapidly disseminate germplasm. We have acclimatized hundreds of plants from large vessels to greenhouse conditions without any loss.

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Table 1. Biomass (g) per 180 ml vessel at each harvest period is shown for 9 combinations of BA and MeJA. Best fits of changes in biomass by time are denoted as either exponential (E) or linear (L).

	Treatment (μM)		Weeks						
Response	BA	MeJa	1	2	3	4	5	6	Fit Type
FW Shoot	0.00	0	0.202	0.425	1.251	1.839	1.916	3.798	E

	0.00	5	0.121	0.476	0.658	1.606	2.121	1.894	E
	0.00	16	0.091	0.420	0.379	0.327	1.144	2.718	E
	0.32	0	0.174	0.602	1.071	1.890	2.535	3.733	E
	0.32	5	0.205	0.250	0.648	1.281	1.851	3.500	E
	0.32	16	0.148	0.137	0.328	0.819	0.856	1.516	E
	1.00	0	0.148	0.325	1.188	2.052	1.895	3.560	E
	1.00	5	0.113	0.202	0.746	0.640	1.319	2.842	E
	1.00	16	0.095	0.215	0.236	0.581	0.921	1.624	E
FW Root	0.00	0	0.018	0.078	0.287	0.994	1.153	2.464	E
	0.00	5	0.025	0.098	0.323	1.013	1.695	2.601	E
	0.00	16	0.044	0.135	0.121	0.210	0.971	2.433	E
	0.32	0	0.051	0.212	0.391	1.169	2.184	4.200	E
	0.32	5	0.027	0.104	0.206	0.853	1.580	3.870	E
	0.32	16	0.019	0.087	0.127	0.563	0.621	1.859	E
	1.00	0	0.025	0.080	0.701	1.319	1.714	3.304	E
	1.00	5	0.029	0.193	0.450	0.404	0.949	3.727	E
	1.00	16	0.025	0.116	0.077	0.198	0.501	1.710	E
FW Rhizome	0.00	0	0.645	1.217	1.738	2.019	2.091	2.866	L
	0.00	5	0.774	1.400	2.144	2.768	2.973	3.190	L
	0.00	16	0.723	1.513	1.934	1.929	2.438	3.853	L
	0.32	0	0.966	2.009	1.987	2.492	3.026	4.082	L
	0.32	5	0.961	1.559	2.226	2.763	3.480	3.726	L

	0.32	16	0.608	1.353	1.639	2.470	2.640	2.916	L
	1.00	0	0.982	1.455	2.640	2.545	2.227	3.932	L
	1.00	5	0.703	1.949	2.627	2.157	2.855	4.410	L
	1.00	16	0.579	1.138	1.033	1.865	3.141	3.255	L
DW Shoot	0.00	0	0.032	0.032	0.088	0.119	0.162	0.293	E
	0.00	5	0.040	0.040	0.051	0.114	0.195	0.152	E
	0.00	16	0.035	0.035	0.036	0.030	0.089	0.198	E
	0.32	0	0.046	0.046	0.078	0.131	0.151	0.275	E
	0.32	5	0.018	0.018	0.055	0.097	0.146	0.257	E
	0.32	16	0.010	0.010	0.029	0.065	0.078	0.120	E
	1.00	0	0.026	0.026	0.082	0.141	0.130	0.257	E
	1.00	5	0.017	0.017	0.058	0.055	0.101	0.201	E
	1.00	16	0.011	0.020	0.022	0.053	0.074	0.126	E
DW Root	0.00	0	0.008	0.008	0.021	0.064	0.078	0.124	E
	0.00	5	0.010	0.010	0.023	0.062	0.127	0.161	E
	0.00	16	0.011	0.011	0.011	0.018	0.096	0.144	E
	0.32	0	0.016	0.016	0.029	0.068	0.159	0.164	E
	0.32	5	0.010	0.010	0.020	0.056	0.128	0.185	E
	0.32	16	0.010	0.010	0.012	0.040	0.056	0.090	E
	1.00	0	0.006	0.006	0.055	0.080	0.115	0.171	E
	1.00	5	0.018	0.018	0.031	0.026	0.101	0.167	E
	1.00	16	0.011	0.011	0.008	0.015	0.044	0.109	E
DW	0.00	0	0.063	0.123	0.173	0.226	0.223	0.300	L

Rhizome									
	0.00	5	0.076	0.143	0.219	0.210	0.297	0.362	L
	0.00	16	0.070	0.162	0.191	0.212	0.278	0.412	L
	0.32	0	0.091	0.213	0.190	0.232	0.281	0.403	L
	0.32	5	0.085	0.161	0.227	0.295	0.363	0.344	L
	0.32	16	0.062	0.151	0.161	0.246	0.265	0.309	L
	1.00	0	0.093	0.146	0.297	0.267	0.216	0.390	L
	1.00	5	0.075	0.218	0.274	0.241	0.298	0.428	L
	1.00	16	0.066	0.120	0.105	0.197	0.312	0.334	L
FW Total	0.00	0	0.865	1.721	3.277	4.852	5.160	9.127	E
	0.00	5	0.920	1.974	3.124	5.386	6.788	7.684	E
	0.00	16	0.857	2.068	2.434	2.466	4.553	9.004	E
	0.32	0	1.191	2.823	3.449	5.551	7.746	12.014	E
	0.32	5	1.192	1.913	3.079	4.896	6.911	11.096	E
	0.32	16	0.774	1.577	2.094	3.852	4.116	6.291	E
	1.00	0	1.155	1.859	4.529	5.916	5.836	10.795	E
	1.00	5	0.846	2.344	3.822	3.200	5.123	10.979	E
	1.00	16	0.699	1.469	1.345	2.644	4.564	6.589	E
DW Total	0.00	0	0.103	0.163	0.283	0.408	0.463	0.717	E
	0.00	5	0.126	0.192	0.292	0.385	0.618	0.674	E
	0.00	16	0.116	0.208	0.238	0.260	0.462	0.754	E
	0.32	0	0.152	0.275	0.298	0.431	0.591	0.842	E
	0.32	5	0.113	0.189	0.302	0.448	0.636	0.786	E

0.32	16	0.082	0.170	0.201	0.351	0.399	0.519	E
1.00	0	0.125	0.178	0.433	0.488	0.461	0.818	E
1.00	5	0.109	0.252	0.363	0.322	0.500	0.796	E
1.00	16	0.087	0.150	0.134	0.265	0.430	0.569	E

Factor	FW Shoot	FW Root	FW Rhizome	DW Shoot	DW Root	DW Rhizome	FW Total	DW Total
MeJa	*	*	*	*	*	*	*	*
BA		*	*	*	*	*	*	
MeJA*BA	*	*	*	*	*	*	*	*

* denotes cell significance at $\alpha = 0.01$

Figure 1. Rocker Figure. Large vessels at time of harvest on rocker-platform (left) and contents of large vessels immediately after harvest (right).

Figure 2. Whole plant FW (a), leaf FW (b), and root FW (c) of turmeric plantlets from the short-term time course in small vessels over six weeks with 1 µM BA (n=4).

Figure 3. Plantlets from the short-term time course that were grown for six weeks in 1 µM BA and 0, 5, and 16 µM MeJa.

Figure 4. FW and DW of turmeric rhizomes grown in the short-term time course in small vessels for six weeks (n=4). Data presented are those for the 1 µM BA and 0 µM MeJa treatment condition. Each line fit was entirely within the 95% confidence interval (not shown) of the other line.

Figure 5. Whole plant FW and DW (a), rhizome DW (b), and rhizome RDW (c) of turmeric plantlets from the long-term time course (n=2). Data was pooled for PGR's.

Figure 6. Microrhizome development after 14 weeks in the long-term time course (a). Secondary rhizomes were apparent when the surface layers are peeled back, as shown in plants on left. After 23 weeks, shoots were in atrophy and primary and secondary rhizomes were enlarged (b). Most of the plantlets had several secondary rhizomes (c).

Figure 7. The relationship between whole plant DW and sucrose use is shown for the short (n=4) and long-term (n=2) time courses (a). All of the data points from the short-term time course fall within the rectangle at the lower left corner of the scatterplot. This rectangle is enlarged (b) to show adequate resolution for the data from the short-term time course. Sucrose use was nearly directly related to the dry weight in the short-term (g of sugar = 0.15 + 1.81 g DW, $R^2 = 0.94$) and long-term (g of sugar = 0.07 + 1.63 g DW, $R^2 = 0.98$) studies.

Figure 8. Turmeric plantlets that were grown in small jars and large vessels for 6 weeks. Microrhizomes are covered by root and leaf tissue layers and are not readily apparent.

Figure 1.

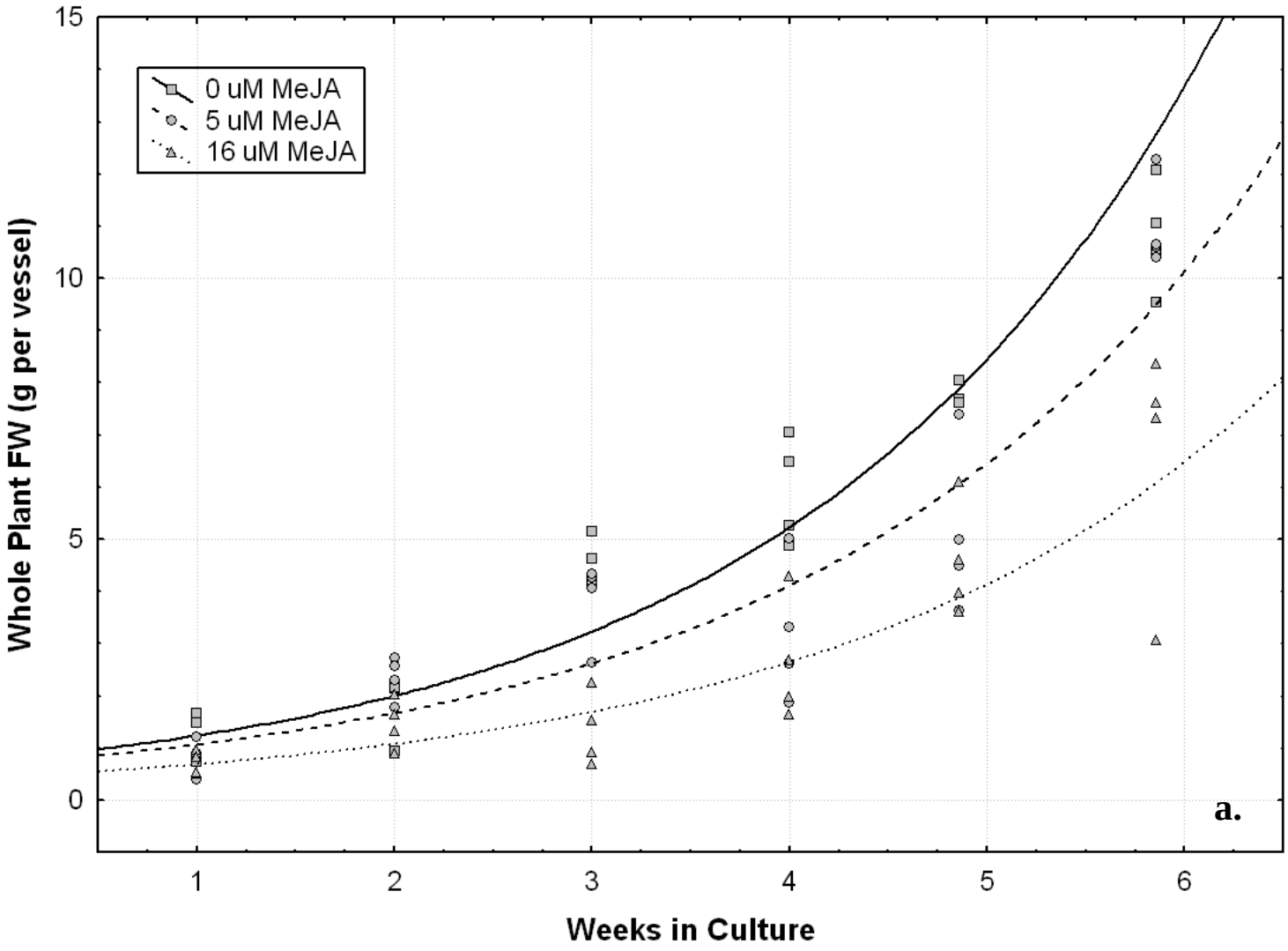
0 uM MeJA
5 uM MeJA
16 uM MeJA
Whole Plant FW (g per vessel)
Weeks in Culture
a.
0
5
10
15
1
2
3
4
5
6

Figure 2.

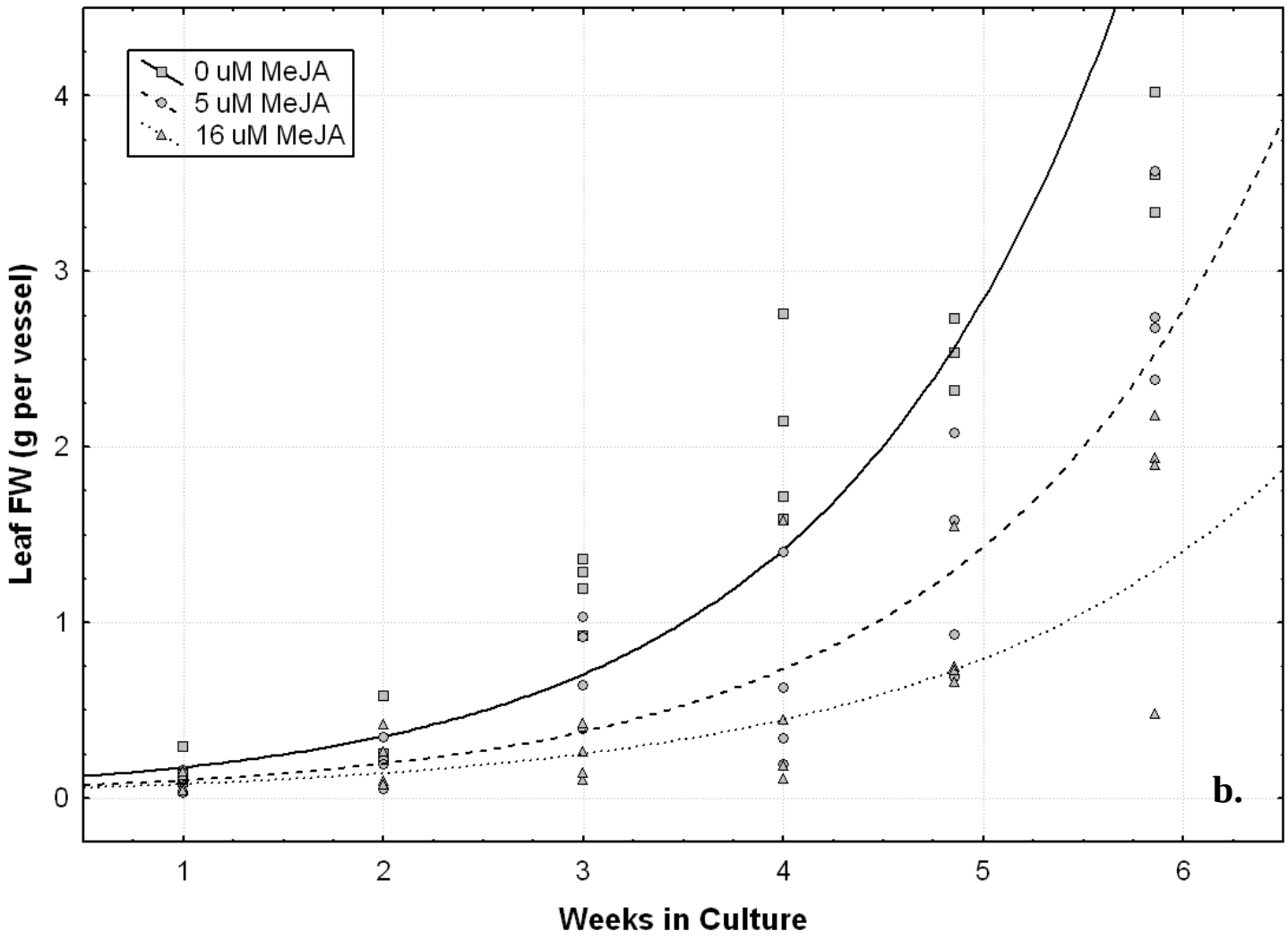
0 uM MeJA
5 uM MeJA
16 uM MeJA
Leaf FW (g per vessel)
Weeks in Culture
b.
0
1
2
3
4
1
2
3
4
5
6

Figure 2.

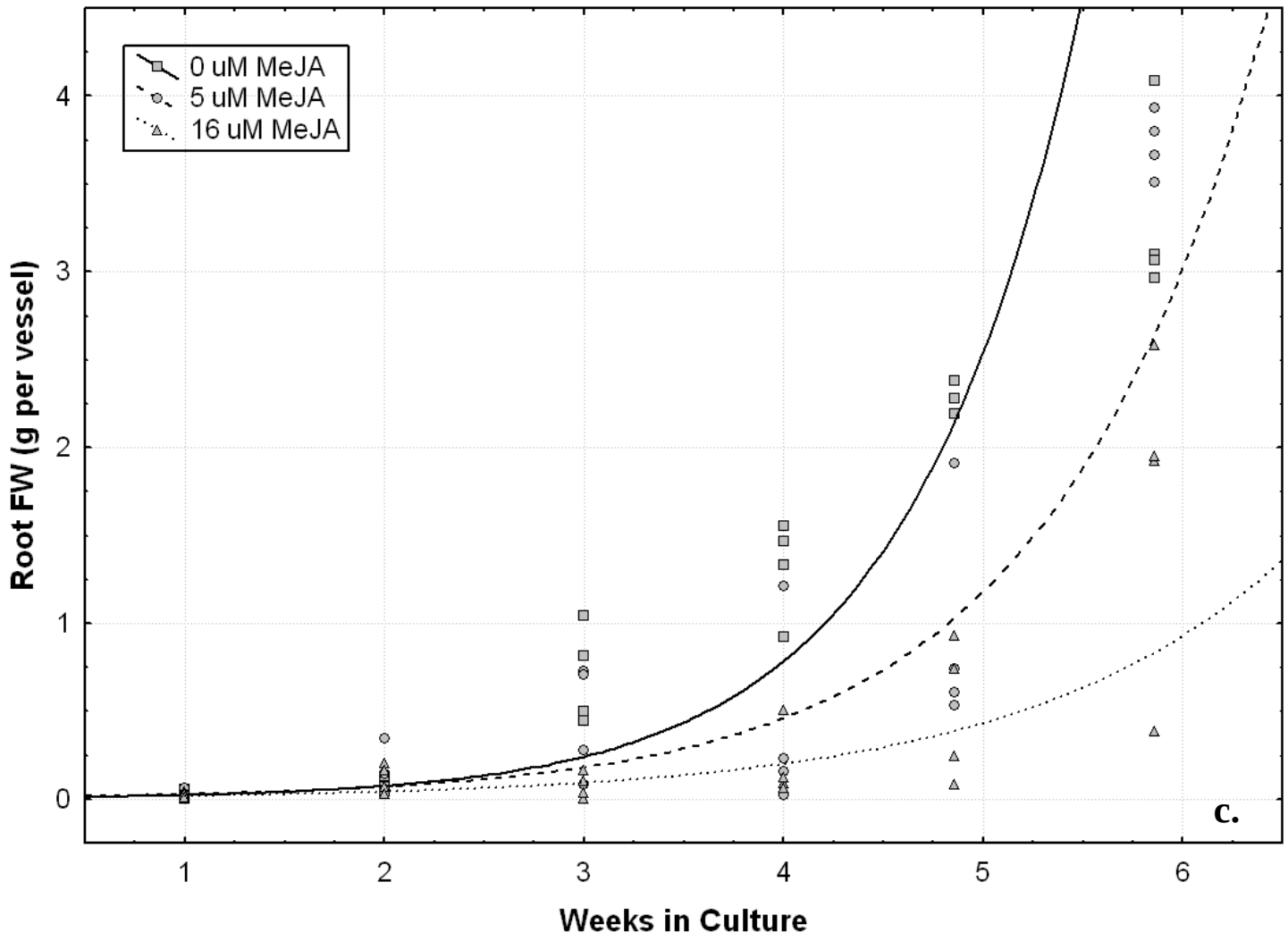
0 uM MeJA
5 uM MeJA
16 uM MeJA
Root FW (g per vessel)
0
1
2
3
4
1
2
3
4
5
6
Weeks in Culture
C.

Figure 2.

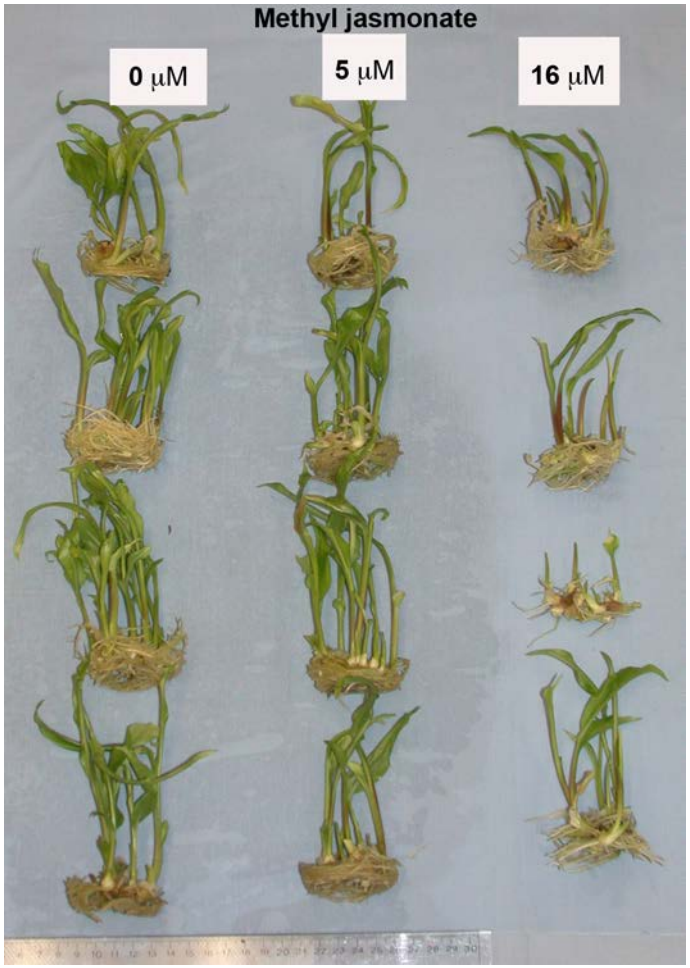

Figure 3.

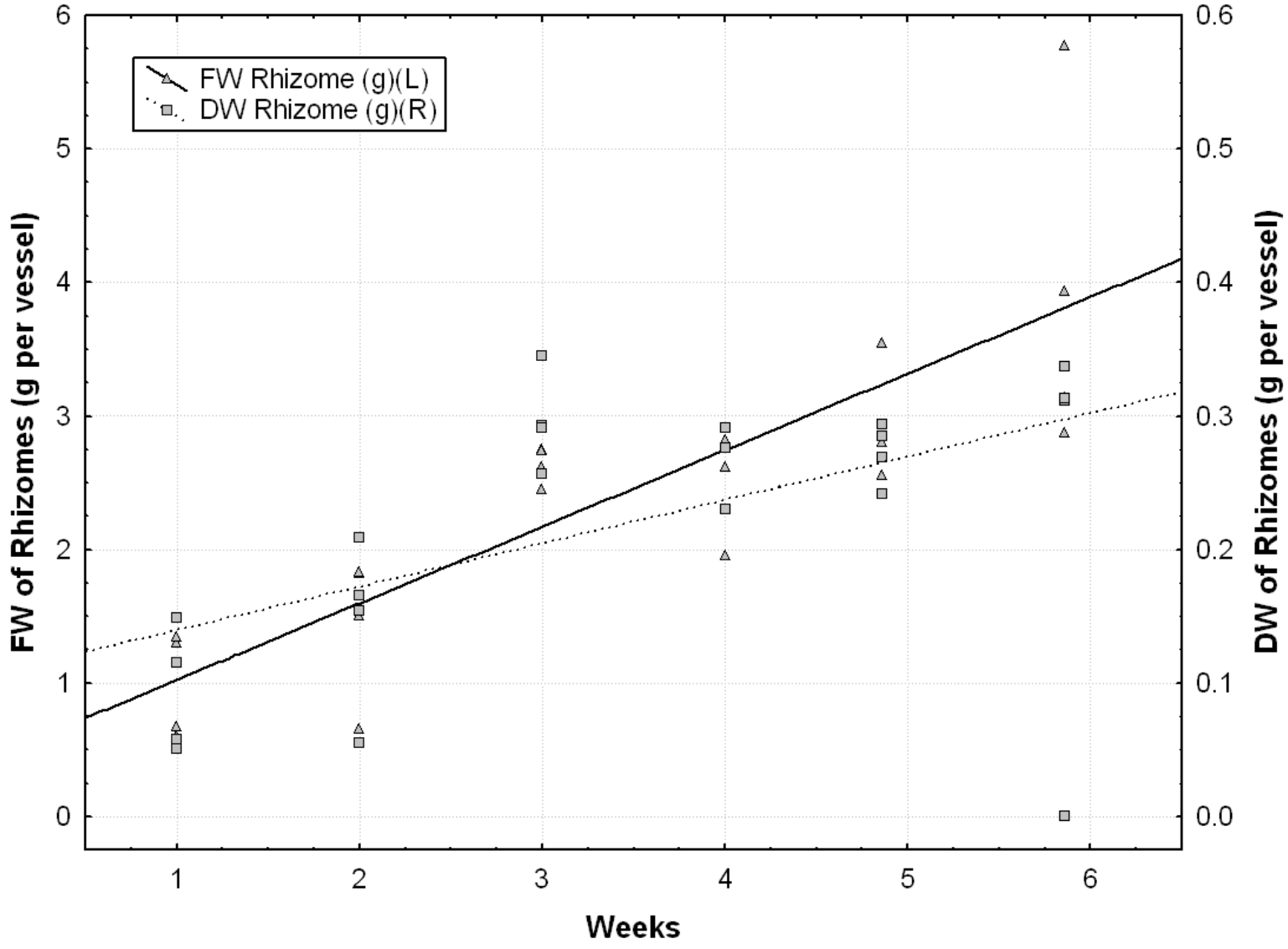
FW Rhizome (g)(L)
DW Rhizome (g)(R)
FW of Rhizomes (g per vessel)
DW of Rhizomes (g per vessel)
Weeks
0
1
2
3
4
5
6
0.0
0.1
0.2
0.3
0.4
0.5
0.6

Figure 4.

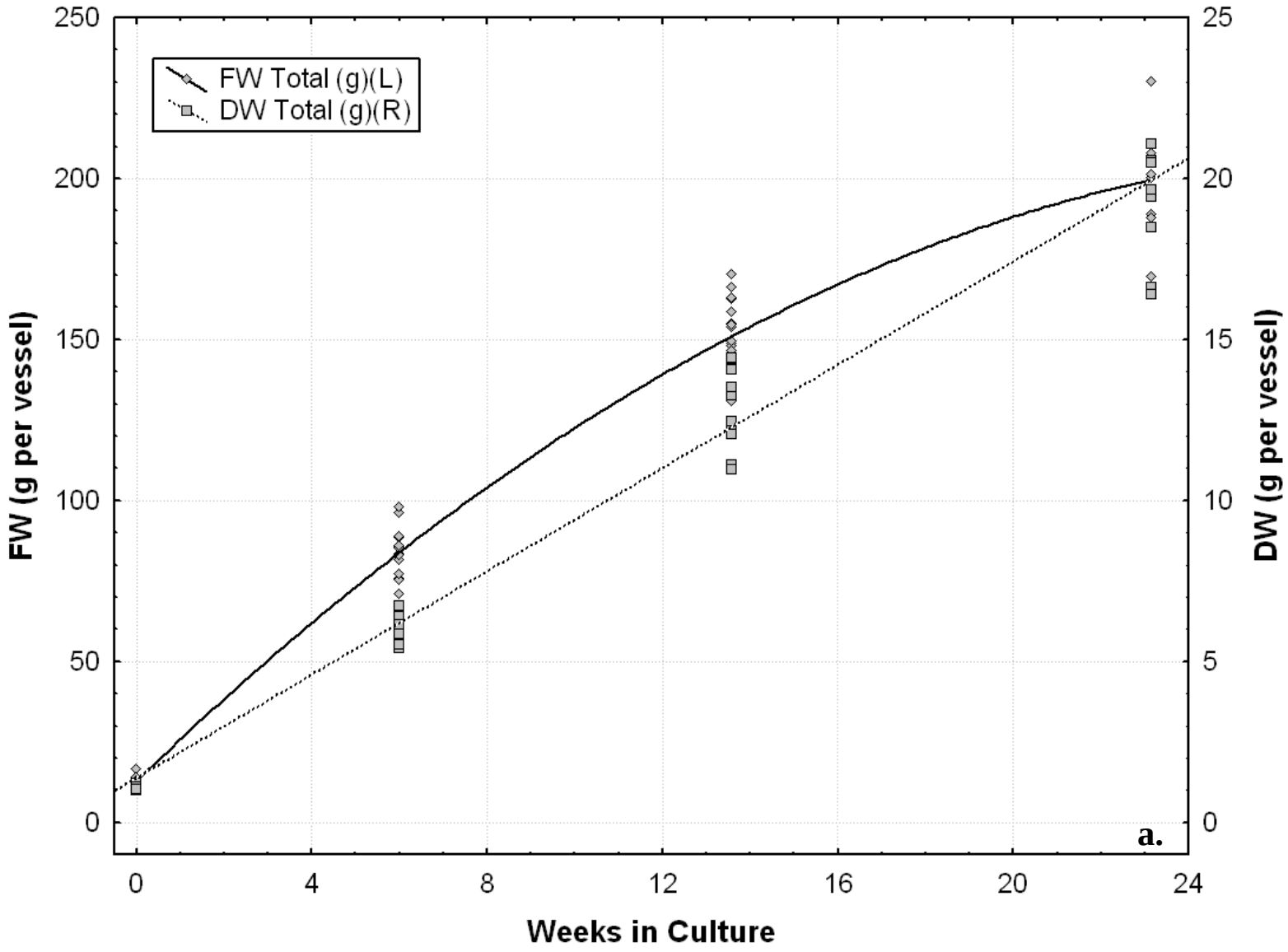
FW Total (g)(L)
DW Total (g)(R)
FW (g per vessel)
DW (g per vessel)
Weeks in Culture
a.
0
50
100
150
200
250
0
5
10
15
20
25
0
4
8
12
16
20
24

Figure 5.

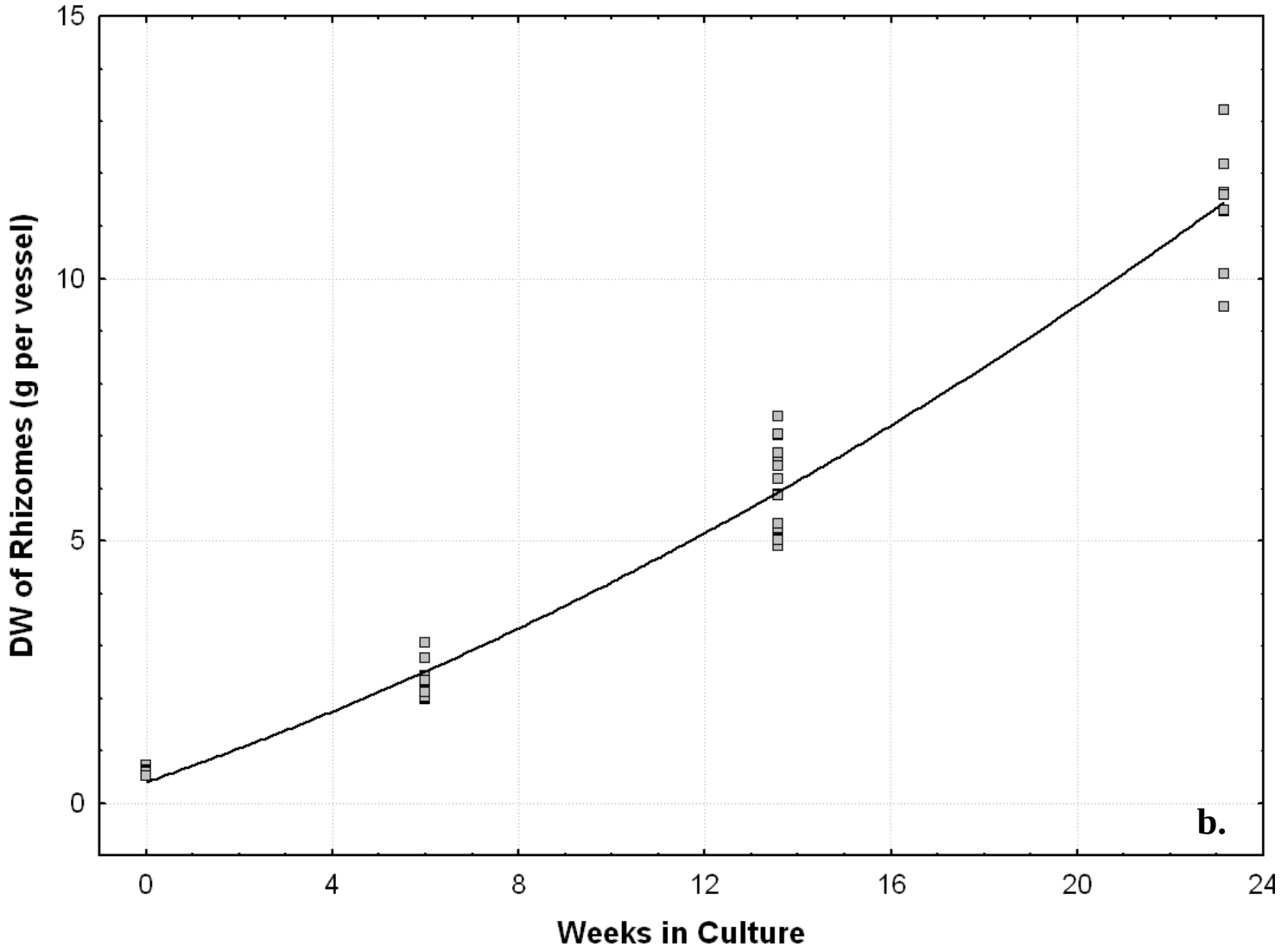
15
10
5
0
DW of Rhizomes (g per vessel)
0
4
8
12
16
20
24
Weeks in Culture
b.

Figure 5.

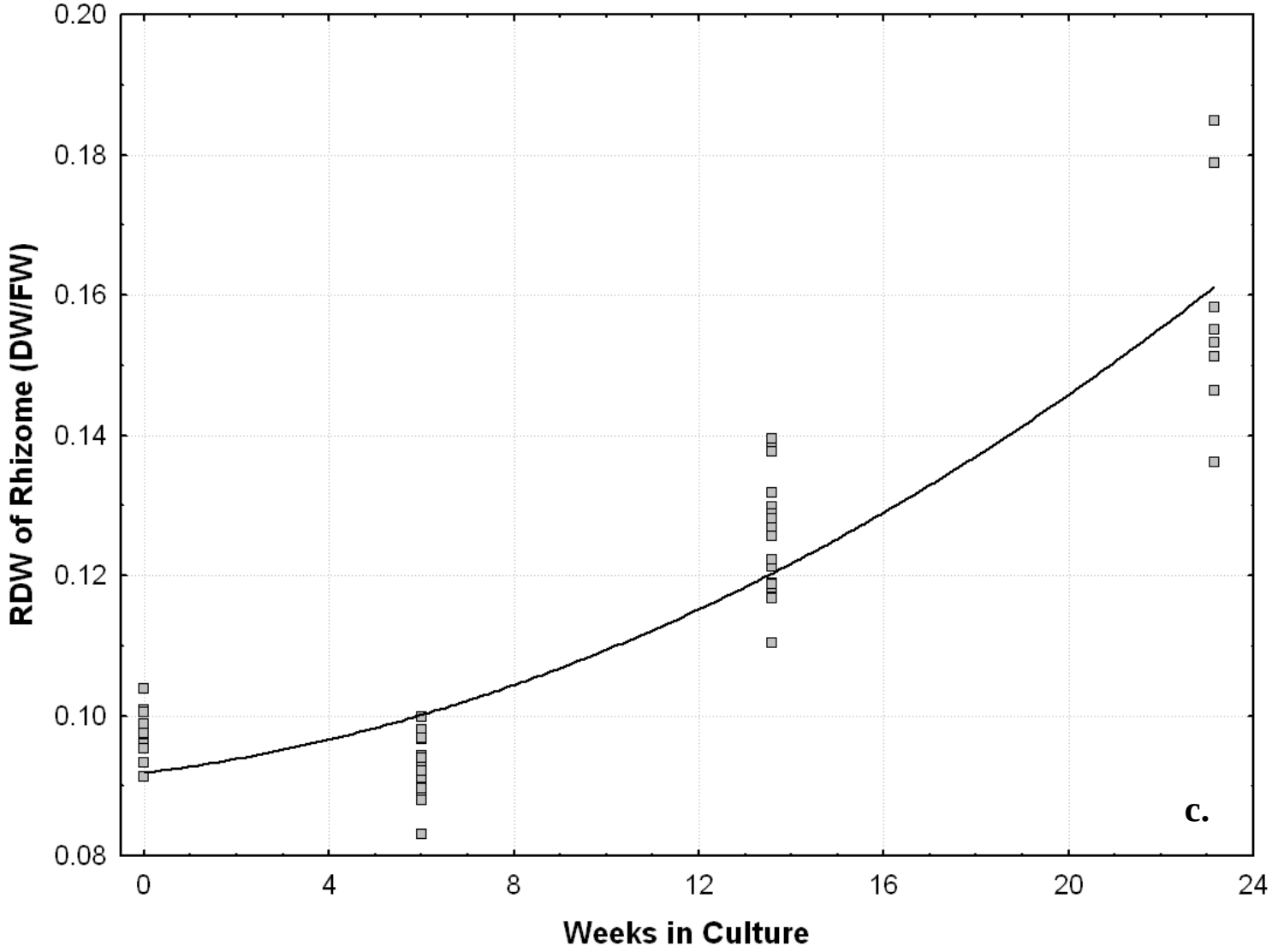
0.20
0.18
0.16
0.14
0.12
0.10
0.08
RDW of Rhizome (DW/FW)
0
4
8
12
16
20
24
Weeks in Culture
c.

Figure 5.

Figure 6.

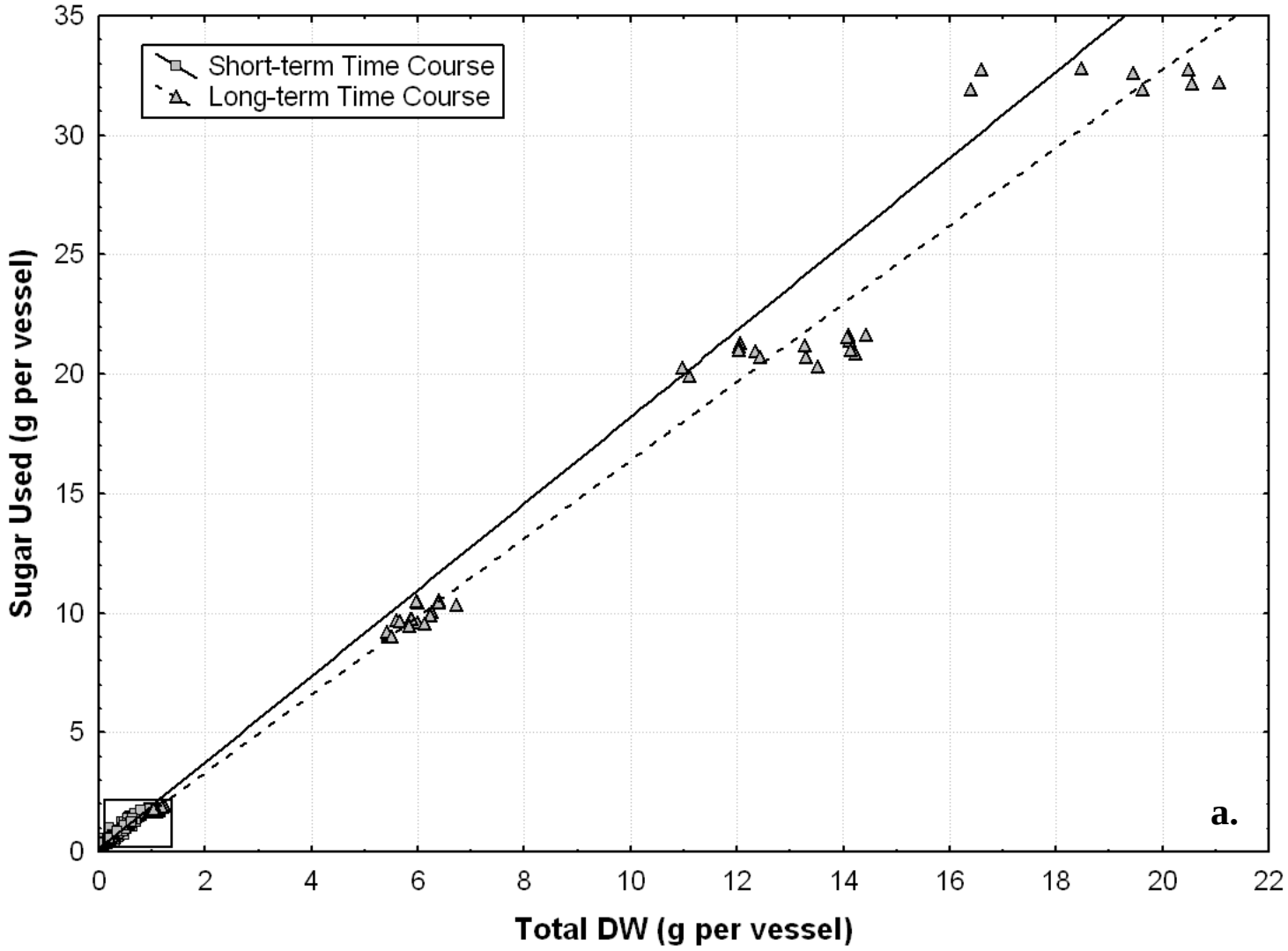
Short-term Time Course
Long-term Time Course
Sugar Used (g per vessel)
Total DW (g per vessel)
a.
0
5
10
15
20
25
30
35
0
2
4
6
8
10
12
14
16
18
20
22

Figure 7.

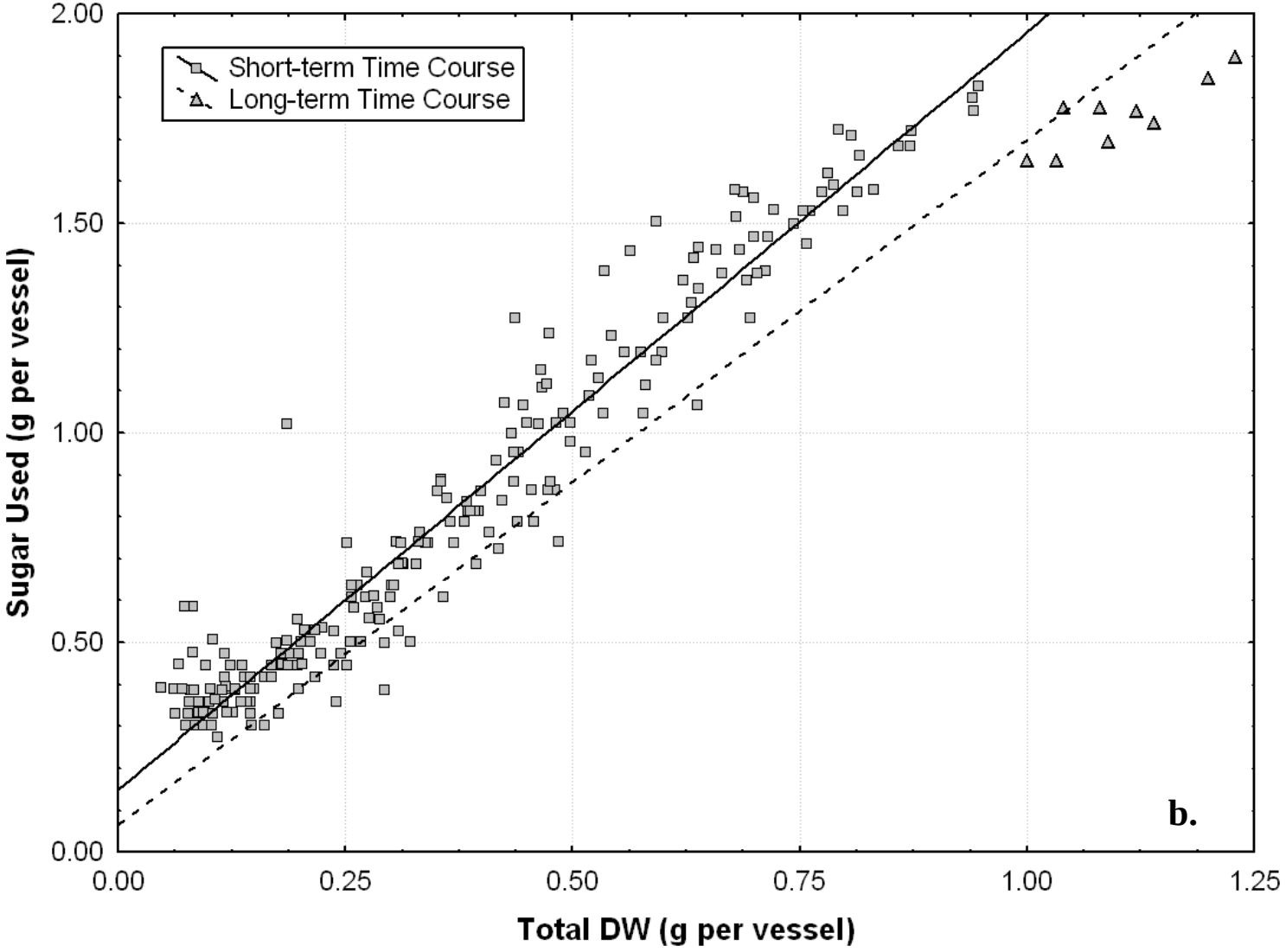
Short-term Time Course
Long-term Time Course
Sugar Used (g per vessel)
Total DW (g per vessel)
2.00
1.50
1.00
0.50
0.00
0.00
0.25
0.50
0.75
1.00
1.25
b.

Figure 7.

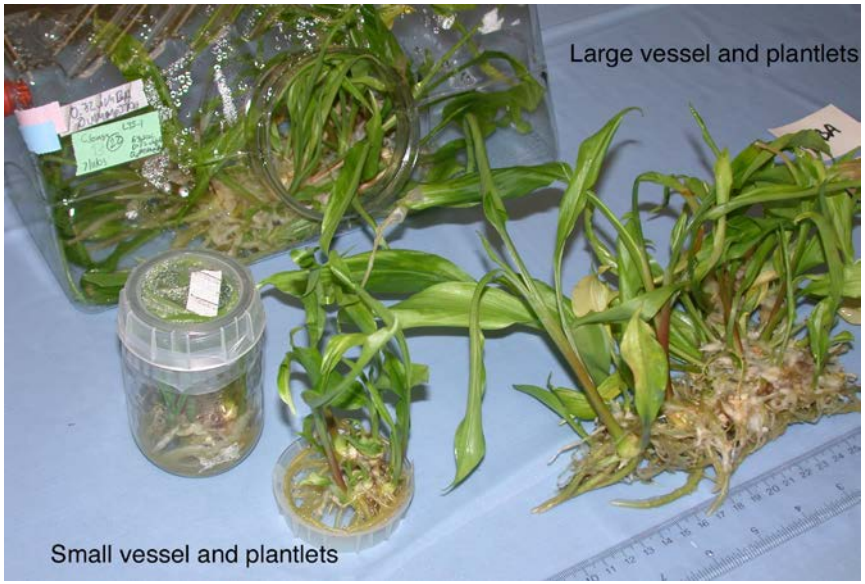

Figure 8.