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Research Report

Optimization of Substrate Formulation and Mineral Nutrition during the Production of Vegetable Seedling Grafts

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Abstract. The use of seedling grafts has been increasingly popular in the production of fruit vegetables in Korea. Superior scion cultivars grafted on seedling rootstocks tolerant to soil-borne diseases often results in higher crop yield and quality. For the production of healthy grafted transplants, seedlings have to be grown under the most favorable cultural conditions. Substrate composition and property as well as mineral nutrition play an important role. Various substrates containing peatmoss, perlite, vermiculite, and coir-dust have been tested for their influence on seedling growth. In the chemical properties of substrates investigated just before grafting and after formation of grafted union, the ECs in 4 substrates rose as the pre-plant fertilizer level was elevated. The ECs in peatmoss containing substrates were lower than those in coir dust containing substrates when all substrates contained equal amount of pre-plant fertilizers. The ECs in substrates containing vermiculite were higher than those in perlite. In all substrates, the elevation of pre-plant fertilizer levels resulted in the increase in NO_3^--N and K^+ concentrations and decrease in Na^+ concentration. The recommended EC levels of substrates for the raising of plug seedlings of bedding plants are applicable to fruit vegetables when the seedlings are grown in peatmoss + perlite (PP) substrate. But those in peatmoss + vermiculite (PV), coir-dust + perlite (CP) and coir-dust + vermiculite (CV) substrates should be altered based on the kinds and rates of substrate components. In case of crops, the water melon rootstock 'Cham Bak' (*Lagenaria siceraria* Standl), showed the stronger salt tolerance than cucumber rootstock 'Heukjong Hobak' (*Cucurbita ficifolia*) indicating that pre- or post-plant fertilizer levels should be varied based on the kinds of crops grown. The peppers grew well in PP and PV substrates rather than CP or CV substrates in before or after grafting. This also implies that the components of substrates should be changed based on the specific crops.

***Additional key words*:** post-plant fertilizer concentrations, pre-plant fertilizer levels, seedling growth

Introduction

The demand for seedling grafted fruit vegetables in Korea has been steadily increasing. According to Ko et al. (2008), about 502 million seedling grafts were used in 2007. They also reported changes in the trend of grafting operation and methodology. Grafting work carried out by growers in the past, however, has shifted recently to group work to facilitate and improve grafting efficiency. The pursuit of improving efficiency changed the method to cut-grafting in which the hypocotyl of the rootstock seedlings are cut off at just above the ground level and used for grafting. When they are stuck in substrate, the successful union between scion and rootstock and the development of new adventitious roots occur simultaneously (Ko et al., 2008; Lee, 1994, 2000). The seedlings with cut-grafting has more vigorous root systems than those of non-cut grafting because formation of new adventitious roots increase the number of primary roots at the base of the cut rootstock hypocotyl with an increased root density per unit volume of soil (Lee, 2000). Plants grown from cut-grafting are also more vigorous and usually more tolerant to drought and cool temperature compared to those grown from regular seedling grafts (Lee, 1994).

For the effective cut-grafting, it is essential to use diseases and insects free substrate with good aeration. Suboptimal substrate conditions often increase the stem decay caused by plant pathogens. Further, in addition, the physical and chemical properties of substrate also influence the success of adventitious root generation and growth of grafted seedlings. Physical properties of substrates should be optimized according to specific needs of the crops grown and container size and shape. This is generally achieved by controlling the constituent materials used to formulate substrate (Bunt, 1988; Nelson, 2003). Chemical properties should also be modified since most of the substrate constituents such as peat moss, coir dust, perlite, and vermiculite have low or unbalanced nutrients (Argo 1998; Choi et al., 2000; Nelson, 2003). This requires the additional pre-plant fertilization during the formulation of substrate enough for the nutrients during the early growth of seedlings after germination and transplanting. The extent of pre-plant fertilizers incorporated into substrates during formulation for plug culture can be found in a number of previous works (Argo, 1998; Koranski, 1990; Nelson et al., 1996; Styer and Koranski, 1997).

However, most of the previous reports are based on floricultural crops. Literature on substrate formulation and fertility control for the grafted plug seedlings of fruit vegetables is not readily available. The seedlings of fruit vegetables grow faster and generally produce more above-ground biomass than those of the floral crops floricultural crops in a given period. Therefore, the optimum pre-plant fertilizer levels for cut grafted seedlings of fruit vegetables could be different to those recommended for general plug seedlings of floricultural crops. The objective of this research was to investigate the effect of substrate formulation and fertilizer conditions on the changes in chemical properties of substrates and the seedling growth during the production of plug-grown seedling grafts of some fruit vegetables.

Materials and Methods

Substrate Formulations and Pre-plant Fertilizer Levels

Canadian Sphagnum peatmoss, coir dust (imported from Sri Lanka), vermiculite, and perlite processed (Shinsung Mineral Corp.) after importing from China were used to formulate substrates. Since most of the commercial substrates have total porosity higher than 85% (Nelson, 2003), we adjusted the blending ratio of the four substrates such as peatmoss:vermiculite (5:5, v/v, PV), peatmoss:perlite (7:3, v/v, PP), coir-dust:vermiculite (5:5, v/v, CV) and coir-dust :perlite (7:3, v/v, CP). The total porosities of the PV, PP, CV, and CP substrates determined before sowing by the method of Fonteno et al. (1981) were 85.5, 87.7, 86.5, and 88.3%, respectively.

To adjust the pH of substrates, the 2.28 $g\cdot L^{-1}$ of dolomitic lime [$CaMg(CO_3)_2$] and 0.63 $g\cdot L^{-1}$ of calcium carbonate ($CaCO_3$) were incorporated into the substrates. For each substrate formulation, 0.9 $mL\cdot L^{-1}$ of soil wetting agent (Aquagro[L], Aquatrols Corp. of the US) was used to help moisturization. The standard fertilizer solution (1.0x) contained 0.303 g of 18-18-18 (N-P_2O_5-K_2O) fertilizer (Omex Agrifluids Co., Ltd. in UK), 3.1 mg of H_3PO_4, 0.015 g of $MgSO_4\cdot 7H_2O$, 3.1 mg of $Na_2B_4O_7$, 3.1 mg of Fe-EDTA, 1.5 mg of Mn-EDTA, 0.9 mg of Zn-EDTA, 0.6 mg of Cu-EDTA, and 0.15 mg of $Na_2MoO_4\cdot 2H_2O$ were dissolved in water and applied on the 1 L of substrate ($mg\cdot L^{-1}$: 190 N, 54.5 P_2O_5, 149 K_2O, 542 CaO, 24.3 MgO and 19.5 S). The 0, 0.5x, 2.0x, and 4.0x treatments contained all of the above fertilizers at 0, a half, two times and four times as much as the standard 1x fertilizer solution.

Seedling Growth before Grafting

The scion and rootstock used for grafting were 'Sweet Pepper' and 'Conesian Hot' for pepper (*Capsicum annuum* L.) 'Joeun Baekdadagi' (*Cucumis sativus* L.) and 'Heukjong Hobak' (*Cucurbita ficifolia*) for cucumber (Seminis Korea Co.) and 'Taepyung Ggul' (*Citrullus vulgaris* Schrad) and 'Cham Bak' (*Lagenaria siceratia* Standl) for watermelon (Nong Woo Seed Co., Korea), respectively.

The seeds of pepper rootstock and scion were sown in to 50-cell (cell volume 68 mL) and 105-cell (30 mL) plug trays, respectively, and both the seeds of rootstock and scion plants in cucumber and water melon in 162-cell (17 mL) plug trays. The seeded plug trays were placed in a germination room with temperature controlled at 27 to 28℃. After cotyledon emergence, trays were transferred to a glass greenhouse maintained at 30℃ (day) and 20℃ (night).

The fertilizers, either 14-0-14 or 20-10-20 (N-P_2O_5-K_2O, Planta Co., CA, USA), were applied at 50 $mg\cdot L^{-1}$ of N after plug stage 2. The fertilizer concentration increased to 120 $mg\cdot L^{-1}$ and 200 $mg\cdot L^{-1}$ of N during the third and fourth plug seedling stages, respectively. Over the entire culture period, the two fertilizer formulations were used weekly in alternation. The leaching rate (volume leached/volume applied) during liquid fertilization and irrigation in all of our experiments were set at 0.3 (Ku and Hershey, 1991). Data on seedling growth and analysis of soil chemical properties were taken 31 days after sowing for pepper and 12 days for cucumber and watermelon.

The all experiments were conducted as completely randomized design with treatments replicated four times (One tray was regarded as a replication).

Seedling Growth during Formation of Graft Union

Substrate formulation and pre-plant fertilizer levels for this experiment were identical to those used for the experiment on seedling growth before grafting stage. The substrates containing the pre-plant nutrient charge were used to fill 50-cell plug trays.

During grafting, the stems of rootstock were cut with a razor blade both at 1 cm above the cotyledon with a 45°

angel and at just above the root zone. The scion seedlings were also cut at 1 cm above the cotyledon with the same angle. The cut scion and rootstock stems were joined together and kept intact using a clip, then seedling grafts were planted into substrate in plug trays. The overall procedure of grafting followed the method graphically described by Lee (1994).

The plug trays were transferred to an acclimatization room for callusing and graft union. The acclimatization room was covered with one layer of black shade cloth which allowed less than 10% transmission of natural light. The relative humidity and temperature inside of the room were controlled to maintain higher than 95% by fogging and 28 to 29℃, respectively. As the time required for adventitious root formation usually takes about 4 days, irradiance inside the chamber is gradually increased from day 5 to day 7 and the seedling grafts were fully exposed to sunlight after 8 days from grafting. Humidity inside the acclimatization room is also gradually decreased in accordance with the time schedule for irradiance to the normal greenhouse condition, but the temperature was controlled to maintain 28 to 29℃. During the experiment the relative humidity inside of the greenhouse was 60 to 70%. Starting from the 8th day on, the seedlings received 200 $mg\cdot L^{-1}$ N in the nutrient solution made with 20-10-20 water soluble fertilizer. The survival rates of seedling grafts during the formation of graft union were close to 100% in all treatments. This is why the data for survival rates of seedling grafts were not presented in this paper.

Data on plant growth and chemical properties of substrates were recorded 13 days after grafting.

The experiment design was completely randomized with 4 replicates of scion/root stock combinations (One tray was regarded as a replication).

Fertility Control after Grafted Seedling Acclimation

The substrates contained equal amounts of pre-plant fertilizers with 1x treatment of previous research were filled into 50-cell plug tray. The grafted seedlings were planted to the plug trays and treated with the same procedure mentioned above. Post-planting fertilization began 8 days after grafting. Four different levels of post-plant nutrient concentration (0, 50, 100, and 200 $mg\cdot L^{-1}$ N) prepared with 14-0-14 and 20-10-20 fertilizers were applied alternatively once a week. The leaching ratio was set at 0.3 (Ku and Hershey, 1991).

The experiment design was completely randomized with 4 replicates of scion/root stock combinations (A tray was regarded as a replication).

Measurement of Seedling Growth and Analysis of Substrates

The pre-grafting growth of rootstock and scion plants of pepper, cucumber and watermelon were measured at 31, 12, and 12 days after sowing, respectively. The growth of seedling grafts during the formation of graft union was determined 13 days after grafting. Measurement on grafted plant growth during acclimation was made at 31, 16, and 30 days, respectively, for pepper, cucumber, and watermelon. The overall procedure for evaluating dry weight of seedlings followed the method described by Choi et al. (2007).

The substrates in individual plant were collected on the same day of the growth measurement and analysis of chemical properties were made. Substrate analysis in each experiment was performed following the saturation extraction method (Warncke, 1986) with the electrical conductivity (EC) measured (Orion-50, Fisher, USA) for extracted solution. Colorimetric analysis was applied to the extracted solution to determine the concentrations of NO_3^--N using a UV/Vis spectrophotometer (CE-5001, Cesil, England) (Cataldo et al., 1975). The concentrations of K and Na in extracted solution were analyzed using an atomic absorption spectrophotometer (AA-680, Shimadzu, Japan).

Statistical Analysis

Data from growth measurements and soil analysis were subjected to a randomized complete block analysis of variance and means were separated by ANOVA and Duncan's multiple range test at $p \leq 0.05$. The growth measurements and soil analysis were also subjected to polynomial regression analysis. We regarded the equation with the highest R^2 and incremental F value between linear and quadratic as the best fit equation, but the equations are not presented in this paper. Data analyses were conducted using CoStat program (CoHort Software V. 6.3, Monterey, CA, USA).

Results

Influence of Substrates and Fertilization on Seedling Growth before Grafting

The dry weights of cucumber rootstock 'Heukjong Hobak' seedlings showed quadratic responses to pre-plant fertilizer levels in CP substrate with the heaviest dry weight produced in 1.0x treatment. The dry weight and electrical conductivity (EC) of the treatments in which seedlings showed the highest growth in each substrate were 293 mg and 1.30 $dS\cdot m^{-1}$ in PP, 243 mg and 3.16 $dS\cdot m^{-1}$ in PV, 248 mg and 2.35 $dS\cdot m^{-1}$ in CP, and 244 mg and 3.78 $dS\cdot m^{-1}$, respectively.

For the dry weight of pepper rootstock 'Conesian Hot' (*Capsicum annuum* L.) seedlings, the elevation in pre-plant fertilizer levels from 0x to 4.0x did not influence the dry weights in four substrates tested. The ECs of 4x treatment in PP, PV, CP and CV substrates were 2.22, 4.26, 5.14 and 6.24 dS·m^{-1}, respectively. However, the response in dry weight to elevate pre-plant fertilizer levels in CP substrate was quadratic with the highest growth in 1.0x treatment. The seedling growth in peatmoss containing substrate such as PP or PV was superior to those in coir dust containing substrates such as CP or CV ($p \leq 0.001$, *F*-test). The seedling growth was the highest in PP substrate followed by PV, CV and CP with significant differences.

The dry weights of water melon rootstock 'Cham Bak' seedlings 12 days after sowing as influenced by pre-plant fertilizer levels showed significant differences among substrates ($p \leq 0.001$) with the highest growth in PP followed by CP, PV, and CV. The dry weight and EC showed the highest growth in each substrate were 135 mg and 2.96

Table 1. Substrate chemical properties and dry weights of rootstock plants 12, 31, and 12 days after sowing of cucumber, green pepper, and watermelon, respectively, as influenced by substrates (S) and pre-plant fertilizer levels (PPFL).[z]

S[y]	PPFL[x]	Cucumber root stock					Green pepper root stock					Watermelon root stock				
		EC (dS·m^{-1})	NO_3^--N (mg·L^{-1})	K^+ (mg·L^{-1})	Na^+ (mg·L^{-1})	Dry weight (mg/plant)	EC (dS·m^{-1})	NO_3^--N (mg·L^{-1})	K^+ (mg·L^{-1})	Na^+ (mg·L^{-1})	Dry weight (mg/plant)	EC (dS·m^{-1})	NO_3^--N (mg·L^{-1})	K^+ (mg·L^{-1})	Na^+ (mg·L^{-1})	Dry weight (mg/plant)
PP	0	1.24	60	63	220	244	1.24	60	63	220	37	2.60	138	75	200	99
	0.5	1.30	146	32	138	293	1.31	87	30	387	142	2.89	217	88	199	111
	1	1.47	168	29	148	221	1.85	136	33	168	123	2.96	326	89	187	135
	2	1.53	200	29	177	246	2.04	167	37	272	138	3.03	350	92	179	116
	4	2.22	354	42	339	215	2.22	354	42	339	175	3.16	643	91	139	118
PV	0	3.10	280	31	302	202	2.58	295	28	370	62	3.16	153	15	338	72
	0.5	3.16	378	29	378	243	3.00	437	30	403	120	3.64	208	24	335	89
	1	3.70	381	30	370	242	3.37	495	33	396	141	3.68	371	42	300	88
	2	3.71	383	30	392	220	3.74	513	34	390	169	3.70	385	46	262	113
	4	3.76	415	30	412	211	4.26	555	45	297	173	3.79	379	78	205	81
CP	0	2.35	194	55	617	248	2.74	86	305	287	13	2.35	302	33	391	87
	0.5	3.18	234	87	643	213	3.27	101	309	399	56	3.18	306	40	377	90
	1	3.23	380	87	621	176	3.65	110	311	342	82	4.24	376	51	302	124
	2	3.27	524	106	438	201	3.61	194	325	435	76	4.63	380	238	295	108
	4	3.98	566	305	287	187	5.14	318	372	627	59	5.40	566	242	221	94
CV	0	3.78	34	39	451	244	3.60	41	26	350	81	5.78	122	68	822	72
	0.5	4.08	81	37	557	216	3.65	70	30	423	99	6.16	200	76	736	73
	1	4.21	101	37	557	202	3.85	153	64	299	93	6.40	330	75	704	83
	2	4.35	122	44	462	181	5.92	156	80	296	118	6.79	371	80	681	101
	4	4.54	140	46	462	174	6.24	187	85	286	119	7.02	384	124	650	74
F significance																
S		***	***	***	***	*	***	*	***	***	***	***	NS	**	***	***
Regression																
PP		L***	L***	Q***	Q***	NS	L***	L***	Q*	NS	NS	L***	L***	Q***	L***	L*
PV		L***	L*	NS	Q***	NS	L***	L***	L***	L***	NS	Q***	Q***	L***	L***	NS
CP		L***	L***	L***	L***	Q**	L***	L***	L***	L***	Q**	L***	L***	L***	L***	NS
CV		L***	L***	L***	NS	NS	L***	L***	L***	Q**	L*	L**	L***	L***	L***	Q**

[z]Rootstocks: Cucumber, 'Heukjong Hobak' (*Cucurbita ficifolia*); Green pepper, 'Conesian Hot' (*Capsicum annuum* L.); Watermelon, 'Cham Bak' (*Lagenaria siceraria* Standl).

[y]Substrates: PP = Peatmoss + perlite (7:3, v/v), PV = Peatmoss + vermiculite (5:5, v/v), CP = Coir-dust + perlite (7:3, v/v), CV = coir-dust + vermiculite (5:5, v/v).

[x]PPFL: pre-plant fertilizer level; 1.0x treatments contained (1 L of substrates) 0.303 g of 18-18-18, 0.015 g of $MgSO_4 \cdot 7H_2O$, 31 mg of $Na_2B_4O_7$, 3.1 mg of Fe-EDTA, 1.5 mg of Mn-EDTA, 0.9 mg of Zn-EDTA, 0.6 mg of KNO_3, 0.15 mg of $Na_2MoO_4 \cdot H_2O$, 3.1 mg of H_3PO_4, 0.202 g of KNO_3, and 2.28 g of $Ca(NO_3)_2 \cdot 4H_2O$; the 0.5x, 2.0x, and 4.0x fertilizers contained half, two times, and four times as much of 1x treatment, respectively, applied to the substrates. Significance in trends of regression or in *F*-test: ***$P \leq 0.001$; **$P \leq 0.01$; *$P \leq 0.05$; NS = non-significant; L = linear; Q = quadratic.

dS·m^{-1} in PP, 113 mg and 3.79 dS·m^{-1} in PV, 124 mg and 4.24 dS·m^{-1} in CP, and 101 mg and 7.02 dS·m^{-1} in CV.

The seedling growth of scion plants and chemical properties of substrate at 12, 31, and 12 days after sowing of cucumber, pepper, and water melon, respectively, were shown in Table 2. ECs in each substrate 12 days after sowing of cucumber increased as the pre-plant fertilizer levels were elevated. The dry weight of seedlings grown in PP substrate decreased linearly as pre-plant fertilizer levels were elevated. But the tendency and statistical differences in dry weights of seedlings among treatments were not observed when plants were grown in PV, CP, and CV substrates. The dry weight and ECs in each substrate with the highest growth were 79.4 mg and 1.60 dS·m^{-1} in PP, 50.6 mg and 4.62 dS·m^{-1} in PV, 108 mg and 3.54 dS·m^{-1} in CP, and 44.2 mg and 4.46 dS·m^{-1} in CV, respectively.

Table 2. Substrate chemical properties and dry weight of scion plants 12, 31, and 12 days after sowing of cucumber, green pepper, and watermelon, respectively, as influenced by substrates (S) and pre-plant fertilizer levels (PPFL).[z]

S[y]	PPFL[x]	Cucumber scion					Green pepper scion					Watermelon scion				
		EC ($dS\cdot m^{-1}$)	NO_3^--N ($mg\cdot L^{-1}$)	K^+	Na^+	Dry weight (mg/plant)	EC ($dS\cdot m^{-1}$)	NO_3^--N ($mg\cdot L^{-1}$)	K^+	Na^+	Dry weight (mg/plant)	EC ($dS\cdot m^{-1}$)	NO_3^--N ($mg\cdot L^{-1}$)	K^+	Na^+	Dry weight (mg/plant)
PP	0	1.60	271	41	125	79.4	0.87	14.4	15	92	38.6	2.49	243	53	665	37.8
	0.5	1.72	273	39	131	55.8	1.03	29.7	16	106	75.8	2.49	260	58	353	32.2
	1	1.74	298	41	102	56.7	1.09	40.4	18	126	92.2	2.83	311	70	341	35.6
	2	1.83	358	40	142	53.1	1.34	53.6	20	129	112.2	3.39	348	74	279	41.4
	4	2.13	496	49	169	37.2	1.42	203.2	35	193	92.2	3.36	377	90	258	37.8
PV	0	3.75	419	32	645	43.6	1.55	27.6	10	217	59.4	1.33	217	19	335	34.2
	0.5	4.21	450	27	741	43.1	1.55	48.6	10	265	104.7	2.36	386	20	338	39.2
	1	4.33	493	30	599	46.4	1.58	51.6	12	203	127.5	3.04	439	42	300	34.7
	2	4.55	511	33	524	47.8	1.86	52.7	14	227	140.3	3.38	442	46	260	34.2
	4	4.62	556	37	481	50.6	2.35	405.0	15	192	129.7	3.51	557	78	226	36.9
CP	0	3.54	202	296	271	77.5	2.16	4.8	112	182	13.3	1.47	51	49	464	31.1
	0.5	3.93	206	272	231	67.8	3.63	8.0	156	159	35.6	1.87	54	234	303	33.6
	1	3.94	303	275	262	44.4	2.74	16.0	153	194	50.0	4.24	302	307	260	34.7
	2	4.23	319	324	238	42.5	2.85	18.8	173	218	53.1	4.63	306	382	215	37.8
	4	4.75	361	342	250	48.6	2.96	205.3	217	237	60.0	5.40	376	434	198	35.8
CV	0	3.98	84	48	682	36.9	1.62	5.4	43	295	40.6	5.78	214	34	961	26.4
	0.5	4.46	101	39	616	44.2	1.80	7.8	40	237	85.8	6.16	271	66	739	27.5
	1	4.63	115	47	508	42.5	2.58	7.7	35	227	103.3	5.79	359	71	721	40.0
	2	4.70	158	51	425	31.1	2.95	8.9	29	210	97.5	6.40	376	73	656	34.4
	4	5.12	401	61	351	40.6	3.14	8.9	24	180	85.0	7.02	384	81	237	28.9
F Significance																
S		***	***	***	***	NS	***	*	***	***	***	***	***	***	***	***
Regression																
PP		L***	L***	Q***	Q**	L***	L***	L***	L***	L***	Q**	L***	L***	L***	L***	NS
PV		L***	L***	L***	L***	NS	L***	L***	L***	L*	Q**	Q***	L***	L***	L***	NS
CP		L***	L***	L***	NS	NS	NS	L***	L***	L***	L***	Q***	L***	L***	Q***	NS
CV		L***	L***	L***	L***	NS	L***	L*	L***	L***	Q***	Q***	L***	Q***	L***	NS

[z]Scion plants: Cucumber, 'Joeun Baekdadagi' (*Cucumis sativus* L.); Green pepper, 'Sweet Pepper' (*Capsicum annuum* L.); Watermelon, 'Taepyung Gggul' (*Citrullus vulgaris* Schrad).

[y]Substrates: PP = Peatmoss + perlite (7:3, v/v), PV = Peatmoss + vermiculite (5:5, v/v), CP = Coir-dust + perlite (7:3, v/v), CV = coir-dust + vermiculite (5:5, v/v).

[x]PPFL: pre-plant fertilizer level; 1.0x treatments contained (1 L of substrates) 0.303 g of 18-18-18, 0.015 g of $MgSO_4\cdot 7H_2O$, 31 mg of $Na_2B_4O_7$, 3.1 mg of Fe-EDTA, 1.5 mg of Mn-EDTA, 0.9 mg of Zn-EDTA, 0.6 mg of KNO_3, 0.15 mg of $Na_2MoO_4\cdot H_2O$, 3.1 mg of H_3PO_4, 0.202 g of KNO_3, and 2.28 g of $Ca(NO_3)_2\cdot 4H_2O$; the 0.5x, 2.0x, and 4.0x fertilizers contained half, two times, and four times as much of 1x treatment, respectively, applied to the substrates. Significance in trends of regression or in *F*-test: ***$P \leq 0.001$; **$P \leq 0.01$; *$P \leq 0.05$; NS = nonsignificant, L = linear, Q = quadratic.

The growth responses of pepper scion seedlings to the elevated pre-plant fertilizer levels in PP, PV and CV substrates showed quadratic response with the heaviest dry weights produced in 2.0x, 2.0x and 1.0x treatments, respectively. The dry weight in CP substrate increased linearly as pre-planting fertilizer levels were elevated. The statistical differences in dry weights were significant ($p \leq 0.001$) among substrates with the greatest growth in PV followed by PP, CV and CP. The ECs of the treatments in which dry weights were the heaviest were 1.34, 1.86, 2.96, and 2.58 $dS \cdot m^{-1}$ in PP, PV, CP, and CV substrates, respectively.

The dry weights and ECs in treatments of the highest growth of watermelon in each substrate were 41.4 mg and 3.39 $dS \cdot m^{-1}$ in 2.0x treatment with PP, 39.2 mg and 2.36 $dS \cdot m^{-1}$ in 0.5x treatment with PV, 37.8 mg and 5.40 $dS \cdot m^{-1}$ in 4.0x treatment with CP, and 40.0 mg and 6.40 $dS \cdot m^{-1}$ in 2.0x treatment with CP, respectively. However, no significant statistical differences in dry weights of each substrate were

Table 3. Substrate chemical properties and dry weights of cucumber and green pepper plants grown in 50-cell pack trays and measured 13 days after cut grafting as influenced by substrates (S) and pre-plant fertilization levels (PPFL).[z]

S[y]	PPFL[x]	Grafted cucumber					Grafted green pepper				
		EC ($dS \cdot m^{-1}$)	NO_3^--N ($mg \cdot L^{-1}$)	K^+ ($mg \cdot L^{-1}$)	Na^+ ($mg \cdot L^{-1}$)	Dry weight (mg/plant)	EC ($dS \cdot m^{-1}$)	NO_3^--N ($mg \cdot L^{-1}$)	K^+ ($mg \cdot L^{-1}$)	Na^+ ($mg \cdot L^{-1}$)	Dry weight (mg/plant)
PP	0	1.06	32	43	109	187	0.85	27	16	24	120
	0.5	1.12	148	49	184	195	1.18	45	18	37	133
	1	1.29	221	51	99	188	1.36	83	21	40	154
	2	1.60	219	53	125	187	1.62	198	38	37	171
	4	2.26	274	54	119	121	1.99	264	46	42	192
PV	0	1.56	84	21	66	207	1.56	41	18	20	119
	0.5	2.03	321	25	89	194	1.73	84	21	53	155
	1	2.15	359	29	123	197	1.64	150	25	66	197
	2	2.48	383	29	121	190	2.58	245	26	62	206
	4	3.14	456	39	133	187	2.68	272	28	89	231
CP	0	3.91	149	46	487	170	2.75	13	193	113	104
	0.5	4.44	194	245	485	175	3.81	46	227	184	103
	1	4.73	210	260	462	156	4.14	89	239	136	174
	2	4.92	212	241	411	161	4.50	180	292	157	151
	4	5.47	241	298	365	168	4.53	181	321	141	122
CV	0	1.85	212	31	277	197	4.47	15	59	280	94
	0.5	2.99	249	33	255	196	5.28	64	68	268	120
	1	3.01	283	41	225	189	5.84	107	63	215	135
	2	3.14	351	51	213	170	6.39	162	86	201	164
	4	3.64	534	81	184	170	6.82	336	96	184	130
F Significance											
S		***	***	***	***	NS	***	***	***	***	***
Regression											
PP		L***	L***	Q***	NS	NS	L***	L***	L***	Q**	L*
PV		L***	L***	L***	L***	NS	L***	L***	L***	L***	L***
CP		L***	L***	L**	L***	NS	L***	L***	L***	NS	Q*
CV		Q***	L***	L***	L***	Q**	L***	L***	Q***	L***	Q***

[z]Seedling grafts (scion/rootstock): Cucumber, 'Joeun Baekdadagi'/'Heukjong Hobak'; Green pepper, 'Sweet Pepper'/'Conesian Hot'.
[y]Substrates: PP = peatmoss + perlite (7:3, v/v); PV = peatmoss + vermiculite (5:5, v/v); PP = coir-dust + perlite (7:3, v/v); CV = coir-dust + vermiculite (5:5, v/v).
[x]PPFL(pre-plant fertilizer level): 1.0x treatment contained (per L of substrates) 0.303 g of 18-18-18, 0.015 g of $MgSO_4 \cdot 7H_2O$, 31 mg of $Na_2B_4O_7$, 3.1 mg of Fe-EDTA, 1.5 mg of Mn-EDTA, 0.9 mg of Zn-EDTA, 0.6 mg of KNO_3, 0.15 mg of $Na_2MoO_4 \cdot H_2O$, 3.1 mg of H_3PO_4, 0.202 g of KNO_3, and 2.28 g of $Ca(NO_3)_2 \cdot 4H_2O$; 0.5x, 2.0x, and 4.0x fertilizers with half, two times, and four times as much as 1x treatment, respectively, applied to substrates. Significance in trends of regression or in *F*-test: ***$P \leq 0.001$; **$P \leq 0.01$; *$P \leq 0.05$; NS = nonsignificant, L = linear, Q = quadratic.

observed among the treatments of pre-plant fertilizer levels.

In the chemical properties of substrates determined prior to grafting, the ECs in 4 substrates rose as the pre-plant fertilizer levels were elevated. The ECs in peatmoss containing substrates such as PP and PV were lower than those in coir-dust containing substrates such as CP and CV when all substrates contained equal amounts of pre-plant fertilizers. The ECs in substrates containing vermiculite were higher than those in perlite. In all substrates, the elevation of pre-plant fertilizer levels resulted in an increase in NO_3^--N and K^+ concentrations and decrease in Na^+ concentration when substrates were analyzed prior to grafting.

Influence of Substrates and Fertilization on Growth of Grafted Seedlings during the Formation of Graft Union and Adventitious Roots

The growth characteristics of grafted cucumber and green pepper seedlings and the chemical properties of substrate 13 days after cut grafting were shown in Table 3. The cucumber seedling grafts in PP substrate showed quadratic responses to elevated pre-plant fertilizer levels with the highest dry weight produced in 0.5x treatment. The dry weight of seedling grafts in PV substrate slightly decreased as pre-plant fertilizer levels were elevated. The greatest dry weights of seedling grafts in CP and CV substrates were 175 mg in 0.5x and 197 mg in 0x treatments, respectively, which were the heaviest among treatments in each substrate ($p \leq 0.05$). The ECs of the treatments in which the dry weight were heaviest in each substrate were 1.12 $dS \cdot m^{-1}$ in 0.5x with PP, 1.56 $dS \cdot m^{-1}$ in 0x with PV, 4.44 $dS \cdot m^{-1}$ in 0.5x with CP and 0.85 $dS \cdot m^{-1}$ in 0x treatment with CV substrate.

The dry weights of seedling grafts of pepper showed a different tendency compared to those of cucumber. When seedling grafts of peppers were grown in PP and PV substrates, the dry weights at 12 days after cut grafting were the heaviest in 4x treatments and the ECs in the two substrates were 1.99 and 2.68 $dS \cdot m^{-1}$, respectively. The dry weight differences for seedling grafts in the two substrates were significantly increased ($p \leq 0.01$) as pre-plant fertilizer levels were elevated. The heaviest dry weights in seedling grafts of peppers grown in CP and CV were 164 mg in 2.0x and 174 mg in 1.0x treatments, respectively. The differences in dry weights of CP and CV substrates were significant ($p \leq 0.001$) and the responses to elevated pre-plant fertilizer levels were quadratic in both substrates.

The ECs of CP or CV substrates 10 days after grafting of cucumber and pepper were significantly higher than those of PP or PV substrates. In the case of CV substrate, the EC in 4.0x treatment was extremely high reaching to 6.82 $dS \cdot m^{-1}$. The reason for high ECs in CV substrate may be due to the leaching out of K^+, Na^+, Cl^- from coir-dust and K^+ and Na^+ from vermiculite as indicated by Choi et al. (2001) and Nelson (2003). The NO_3^--N concentrations increased as the pre-plant fertilizer levels were elevated. In both CP and CV substrates in which the Na^+ concentrations were very high, the PO_4^--P concentrations became lower (data are not shown). The K^+ concentrations in CP and CV substrates which contained coir-dust were much higher than that found in PP and PV substrates indicating that coir-dust released much K^+ into soil sap.

Influence of Substrates and Fertilization on Growth of Grafted Seedlings during Acclimatization

The dry weight of cucumber 16 days after grafting and transplanting into 4 different substrates are shown in Table 4. The highest seedling dry weight and substrate EC were 336 mg and 0.96 $dS \cdot m^{-1}$ in 100 $mg \cdot L^{-1}$ N with PP, 354 mg and 2.21 $dS \cdot m^{-1}$ in 100 $mg \cdot L^{-1}$N with PV, 308 mg and 4.66 $dS \cdot m^{-1}$ in 200 $mg \cdot L^{-1}$ N with CP, and 366 mg and 1.98 $dS \cdot m^{-1}$ in 50 $mg \cdot L^{-1}$ N treatment with CV.

The green pepper 31 days after grafting had the heaviest dry weight in PP substrate followed by PV, CV, and CP substrates. But the EC of the substrates were the highest in CP followed by CV, PV and PP substrates. These results indicate that high soluble salts in CP and CV substrates influenced the growth of pepper seedling grafts negatively.

The dry weights of watermelon 30 days after grafting showed quadratic responses to elevated post-plant fertilizer levels with the highest yield produced in 50 $mg \cdot L^{-1}$ N treatments of all substrates tested. This result suggests that the watermelon is very sensitive to soluble salts compared to green pepper and cucumber. When substrates were compared with the equal amount of post-plant fertilizer applied, dry weights of watermelon grown in perlite containing (PP and CP) substrates were heavier than those grown in vermiculite containing (PV and CV) substrates.

The elevation of fertilizer levels during the acclimation of cucumber, pepper and watermelon seedling grafts in the greenhouse resulted in an increase in NO_3^--N and K^+, but the decrease in Na^+ concentration in the substrates. The NO_3^--N and K^+ concentrations in substrates were directly influenced by the concentration of fertilizers applied. In case of Na^+ concentration, elevation of complete fertilizer concentrations resulted in the increase of K^+ concentrations in substrates.

Discussion

Most substrates used for plug culture contain pre-plant nutrient charge to ensure an optimum seedling growth after germination. Appropriate levels of nutrient charge fertilizers have been reported for plug seedling culture (Argo, 1998; Nelson, 2003). The salinity (EC) of the substrates is generally

Table 4. Substrate chemical properties and dry weight of cucumber, green pepper and watermelon plants grown in 50-cell plug trays for 16, 31, 30 days from as influenced by substrates (S) and post-plant fertilizers concentrations (PPFC) during greenhouse acclimatization.[z]

S[y]	PPFC[x]	Grafted cucumber					Grafted green pepper					Grafted watermelon				
		EC ($dS\cdot m^{-1}$)	NO_3^--N	K^+	Na^+	Dry weight (mg/plant)	EC ($dS\cdot m^{-1}$)	NO_3^--N	K^+	Na^+	Dry weight (mg/plant)	EC ($dS\cdot m^{-1}$)	NO_3^--N	K^+	Na^+	Dry weight (mg/plant)
			($mg\cdot L^{-1}$)					($mg\cdot L^{-1}$)					($mg\cdot L^{-1}$)			
PP	0	0.61	6	161	160	299	0.60	19	15	183	443	1.30	34	115	81	342
	50	0.70	84	151	106	329	0.63	23	18	136	526	1.68	174	164	72	334
	100	1.00	241	210	143	336	0.90	37	23	121	427	1.86	231	165	64	329
	200	2.32	406	255	146	318	0.98	58	23	130	448	2.10	357	186	60	321
PV	0	1.33	129	25	432	284	1.16	44	34	390	420	2.24	712	51	258	261
	50	2.21	335	30	241	324	1.67	252	34	322	426	2.74	453	54	257	251
	100	2.21	520	31	298	354	2.17	289	38	262	455	3.10	313	53	241	288
	200	2.44	518	34	211	338	2.24	303	42	166	381	3.90	135	87	232	275
CP	0	2.92	20	39	313	287	3.10	123	250	382	176	3.07	106	50	350	323
	50	3.27	159	40	334	308	3.84	140	316	311	239	3.41	507	58	340	341
	100	4.66	395	82	409	285	4.53	402	411	280	201	4.06	549	66	307	314
	200	5.61	698	92	456	282	5.37	444	515	223	195	4.32	607	68	292	293
CV	0	1.72	48	28	577	301	2.96	71	33	433	333	1.75	349	104	128	304
	50	1.98	79	28	344	366	3.32	82	32	406	374	2.07	378	130	107	315
	100	2.57	179	41	142	339	3.60	89	40	391	339	2.11	464	142	95	258
	200	2.99	504	89	97	320	3.95	111	45	360	296	2.20	467	133	70	283
F Significance																
S		***	***	***	***	NS	***	***	***	***	***	***	***	***	***	***
Regression																
PP		L***	L***	L***	NS	Q*	L***	L***	L***	Q***	NS	L***	L***	L***	L***	NS
PV		L***	L***	L***	Q**	NS	L***	Q***	L***	L***	NS	L***	L***	Q***	L***	NS
CP		L***	L***	L***	L***	NS	L***	L***	L***	L***	NS	L***	L***	L***	L***	NS
CV		L***	L***	L***	L***	NS	L***	Q*	L***	L***	NS	L***	L***	L***	L***	NS

[z]Seedling grafts (scion/rootstock): Cucumber, 'Joeun Baekdadagi'/'Heukjong Hobak'; Green pepper, 'Sweet Pepper'/'Conesian Hot'/Scion plant; Watermelon, 'Taepyung Ggul' (*Citrullus vulgaris* Schrad)/'Cham Bak' (*Lagenaria siceraria* Standl); Seedling grafts were moved to a greenhouse at 8 days after grafting.
[y]Substrates: PP = Peatmoss + perlite (7:3, v/v), PV = Peatmoss + vermiculite (5:5, v/v), CP = Coir-dust + perlite (7:3, v/v), CV = coir-dust+vermiculite (5:5, v/v); all substrates contained equal amounts of pre-plant fertilizers to 1.0x treatment in Table 1.
[x]Fertilizer concentrations were based on N concentration using14-0-14 and 20-10-20 (N-P_2O_5-K_2O) commercial analysis fertilizers. The seedlings were feed with the fertilizers once a week and two were applied alternatively. Significance in trends of regression or in *F*-test: ***$P \leq 0.001$; **$P \leq 0.01$; *$P \leq 0.05$; NS = nonsignificant, L = linear. Q = quadratic.

controlled to a range of 0.5 to 1.0 $dS\cdot m^{-1}$ (Koranski, 1990; Nelson et al., 1996; Styer and Koranski, 1997). In addition, Koranski (1990) and Styer and Koranski (1997) also reported the appropriate EC levels and individual nutrient concentrations. Based on their claim, the EC values should be in the range between 0.75-1.2 $dS\cdot m^{-1}$ during plug stage 1 (radicle emergence from seed coat) and stage 2 (root penetration into substrate and emergence of cotyledon). The desired EC levels in substrates are 1.0 to 1.5 $dS\cdot m^{-1}$ for the growth of stage 3 (true-leaves emerge and grow) and stage 4 (3-4 true leaves, ready for transplanting and shipping), or holding seedlings.

In comparing ECs of the four substrates used in this study, the formulations containing peatmoss (PP, PV) had lower ECs than those containing coir-dust (CP, CV). The substrate formulations containing vermiculite had also higher ECs than those containing perlite. The significant differences of ECs measured at 12, 31, and 12 days after sowing of rootstock and scion seeds of cucumber, green pepper, and watermelon appear to have occurred by the release of K^+, Na^+ and Cl^- by coir-dust and K^+ and Na^+ from vermiculite as reported by Arena and Varina (2002), Choi et al. (2000), and Nelson (2003).

In considering the influences of substrates and pre-plant fertilizer levels on seedling growth before grafting in our research, the rootstock and scion plants of three crops grew well when the ECs were lower than 3.0 $dS \cdot m^{-1}$ in PP substrate. But the seedling growth based on the dry weight in PV, CP, and CV substrates is acceptable for culture seedling grafts even though their growths were slightly inferior to those in PP substrate. These results indicate that the acceptable ranges of ECs for seedling growths before grafting should be monitored especially when coir-dust is used as a substrate component.

The seedlings of rootstock and scion are usually grafted 10-15 days after sowing for cucumber and watermelon, and 30-35 days after sowing for peppers in Korea. Even though seeds of scion cultivar are usually planted one or two days later than that of rootstocks, we sowed all seeds of the three crops on the same day in this study. But dry weights of the above-ground parts of cucumber and watermelon after 12 days from sowing and those of pepper rootstocks 31 days after sowing were much heavier compared to their scion seedlings when measured after a certain period of time. This implies that rootstock plants have a much higher salt tolerance and grow faster than scion cultivars in cucumber, green pepper, and watermelon. Also, 'Cham Bak' rootstock plants had a much higher salt tolerance than 'Heukjong Hobak' scion plants in watermelon. The dry weight of pepper rootstock 'Conesian Hot' plants grown in CP or CV substrates were significantly decreased compared to those of 'Sweet Pepper' scion seedlings. The rootstock cultivar appears to be more sensitive to high levels of K^+, Na^+, and Cl^- in coir-dust containing substrates compared to the scion cultivar, although peppers in general lack tolerance to high concentrations of these ions.

Substrate EC and pre-plant fertilizer levels influenced the performance of seedling grafts during graft union formation and adventitious root formation. The dry weight of pepper seedling grafts increased as pre-plant fertilizer levels were elevated in PP and PV substrates, but 1.0x in CP and 2.0x in CV provided the highest dry weights among different treatments in each substrate. Trends in the influence of substrate formulations and pre-plant fertilizer levels on seedling growth were similar to those found for seedling growth prior to grafting (Tables 1 and 2). The cucumber has a low salt tolerance and green pepper grew poorly in CP and CV substrates. Therefore, it is necessary that cucumber should be grown in PP, PV, or CV substrates with the ECs lower than 1.85 $dS \cdot m^{-1}$. In case of peppers, PP and PV substrates with the elevation of pre-plant fertilizer levels to 4.0x rather than CP and CV are better for growth of seedling grafts.

During greenhouse acclimatization of seedling graft, the ECs of the PP substrate that provided highest dry weights in all fertilizer levels for cucumber, pepper, and watermelon were 1.00, 0.63, and 1.68 $dS \cdot m^{-1}$, respectively (Table 4). But the ECs of the treatments showing the heaviest dry weights of cucumber, pepper, and watermelon in each substrate were 2.21, 2.17, and 3.10 $dS \cdot m^{-1}$ in PV, 3.21, 3.84, and 3.10 $dS \cdot m^{-1}$ in CP, and 1.98, 3.32, and 2.07 $dS \cdot m^{-1}$ in CV, respectively. These results suggest that that desired EC levels during acclimatization are different by substrate and recommendations for salinity control should be varied by substrate formulations. In this study, the best EC level for producing seedling grafts was the lowest in PP, followed by PV, CV, and CP substrates. Judging from plant response to elevated EC readings, 'Cham Bak' watermelon rootstock plants exhibited higher salt tolerance during seedling growth prior to grafting compared to rootstocks of cucumber and pepper (Table 1). 'Cham Bak' rootstock plants also showed and highest level of salt tolerance during post-grafting growth, indicating that proper EC levels for seedling graft production must be varied by specific crops.

In conclusion, the EC levels and fertilization recommended for producing quality grafted seedlings are similar to those reported for bedding plant plug production when PP substrate is used. However, the salinity levels in PV, CP, and CV substrates could be elevated depending on the used substrate components. 'Cham Bak' watermelon rootstock plants were more salt tolerant than 'Heukjong Hobak' rootstock plants used for cucumber, indicating that pre- or post-plant fertilizer levels need to be adjusted based on the crops grown. The grafted peppers grew better in PP and PV substrates than in CP or CV substrates, suggesting that substrates composition should also be optimized based on specific crops.

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