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Humic Substances Improve Vegetable Seedling Quality and Post-Transplant Yield Performance Under Stress Conditions

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Abstract: Vegetable growers require vigorous transplants in order to reduce the period of transplant shock during early stand establishment. Organic media containing solid humic substances (HS) are amendments that have not been comprehensively explored for applications in containerized vegetable transplant production systems. In this study, HS (1% v/v) were applied to a peat-based growth medium to evaluate pre- and post-transplant growth modulation of four economically important vegetable species. Those were: pepper, tomato, watermelon, and lettuce. Seeding for all species was performed in two periods in order to evaluate their post-transplant yield performance under drought (water deficit vs. well-watered) and heat (hot vs. cool season) stresses. Compared with control, HS-treated plants had: 1) increased leaf and root biomass after transplanting due to faster growth rates; 2) lower root/shoot ratio before transplanting, but higher after 10 days of field establishment; and 3) increased root length and surface area. The negative effects of heat and drought stresses on crop yield were more prominent in control plants, while HS-treated transplants were able to mitigate yield decreases. The results clearly demonstrated the benefits of using solid HS as a management input to improve transplant quality in these crop species.

Keywords: containerized transplants; humic acids; relative growth rate (RGR); specific root length (SRL); heat and drought stresses; heatmaps

1. Introduction

In vegetable production, the use of containerized transplants is a standard practice to establish crops in open fields and protected environments. The advantages of transplants over direct seeding have been recently reviewed by Leskovar [1]: transplanting can optimize the timing and scheduling for field cultivation, shorten the cropping period, increase growth cycles, provide uniform, rapid growth and phenological synchrony (flowering, fruit set), and enhance yield and earliness. However, transplants will inevitably suffer from the mechanical damage of root tips and hairs due to the removal of seedlings from the tray, disturbing the root/shoot balance and causing transplant shock and transiently shoot growth stunting [2,3]. Poorly grown transplants will negatively affect plant performance (or tolerance) in post-field establishment environments which is often accompanied by different abiotic stresses. Therefore, a high-quality transplant should have an ability to bear transient or long-lasting field environmental changes, better survival and uniform stand establishment, and higher resource use efficiency, which will eventually achieve high and profitable yield [4]. Transplants are typically grown in multicell trays. Due to the limited volume of cells and short growing cycle (4 to 6 weeks), transplant quality is often determined by root developmental traits and root-to-shoot balance in the confined cells; high transplant quality is typically associated with vigorous root growth such as higher root length, surface area, and dry weight accumulation [4,5]. For example, lettuce seedlings grown with a proper level of N fertilization (60 mg/L) in the growing media produced better quality transplants with higher root dry weight, and subsequent yield performance as compared with seedlings grown with excessive or low N inputs [6]. It has been recognized that large root systems (represented by biomass) could benefit transplant growth with higher growth rate and improved water and nutrient capture in the soil [2].

Several management factors are known to affect transplant quality (root and shoot developmental traits), such as nitrogen fertilization rate [7], irrigation systems [8], container cell size [9], and light quality [10]. In addition, organic sources such as plant (sesame and alfalfa meal, wood fiber, coconut coir) and animal (fish meal and animal manure)-based compost, and vermicompost, are media amendments that can be potentially used in transplant production due to their potential roles for biostimulation, biofertilization, and plant pathogen suppression [11]. Organic sources can affect germination and emergence rates, and physical and chemical structures of the growth media and rhizosphere shortly after transplanting in the field, which ultimately could be translated into improved plant growth and biomass and early yield. For example, Jack et al. [12] used plant- and animal-based vermicompost (earthworm-driven) and thermogenic compost (self-heating), and found that a small level of additional sesame compost (1–2.5% v/v) in peat-based commercial media significantly increased tomato transplant shoot biomass. However, the use of organic substrates has to be thoroughly tested and validated since certain amended levels could negatively affect seedling growth due to their bound or unbound high salt content [11].

Humic substances (HS), resulting from the decomposition of plant and animal residues, have been widely reported to be used as organic amendments for their biostimulation (auxin-like) effects on enhancing plant root development, nutrient acquisition, and shoot growth [13]. In vegetable transplant production, HS have been used as liquid extractants (humic acids, HA) and applied as foliar sprays. Hartwigsen and Evans [14] used 2.5 and 5 g/kg HA in cucumber and squash seedlings, which resulted in significantly higher root fresh weight and lateral root length; Turkmen et al. [15] used 1 g/kg HA in tomato seedlings, which resulted in improved seedling growth and nutrient contents; Osman and Rady [16] used 0.5 g/L HA as an additive to growing media and found the dry weights, relative water contents, and NPK uptake of tomato and eggplant transplants were all increased. However, no research has been found using solid HS in seedling production. Compared with liquid HS, which can be dissolved easily and normally have quick and profound effects on plant growth [13], solid forms of HS containing humin have less intense effects, but they could increase media water holding capacity due to increased cellulose contents, and nutrient retention due to their cation exchange capacity (CEC) with much longer existence in soil solutions [17–19], which could make them suitable as supplementary amendments for growing media. Therefore, the potential use of solid HS products with the composition of both HA and humin could improve growing media properties and vegetable seedling quality traits, and the beneficial effects on transplants could last longer, even after field establishment.

In this study, we evaluated how and to what extent solid HS added to a peat-based growing media affected root and shoot developmental traits pre- and post-transplanting, as well as subsequent yield of four vegetable species: tomato, pepper, watermelon, and lettuce. We hypothesized that media amended with HS would improve root development and root-to-shoot growth modulation of containerized seedlings during the nursery period (pre-transplanting), as well as long-standing growth during field establishment (post-transplanting), which in turn will increase yield performance.

2. Materials and Methods

2.1. Plant Materials, Growing Media, and Amendment Treatments

We selected four commercial vegetable species representing high-value vegetable crops, each with two distinctive cultivar types (Figure S1): *Capsicum annuum* with cv. Hunter as bell pepper and cv. Jalafuego as jalapeño pepper; *Solanum lycopersicum* with cv. HM1823 as round tomato and cv.

Sakura as cherry tomato; *Citrullus lanatus* with cv. Estrella as diploid (seeded) watermelon and cv. Fascination as triploid (seedless) watermelon; *Lactuca sativa* with cv. Sparx as romaine lettuce and cv. Buttercrunch as butterhead lettuce. Jalafuego, Sakura, Sparx, and Buttercrunch seeds were obtained from Johnny's Selected Seeds (Winslow, ME, USA); Hunter, Estrella, and Fascination from Syngenta (Minneapolis, MN, USA); and HM1823 from Clifton Seed Company (Faison, NC, USA).

Speedling (Ruskin, FL, USA) polystyrene 200-cell trays with inverted pyramid cells (Model TR200A, 2.5 × 2.5 cm^2 × 7.6 cm deep with 32 cm^3 volume per cell) were used for transplant growth in pepper, tomato, and lettuce. Watermelon seeds were sowed into 128-cell trays (Model TR128A, 3.1 × 3.1 cm^2 × 6.4 cm deep with 43 cm^3 volume per cell). Lambert Germination, Plugs and Seedlings (LM-GPS) growing media (90% sphagnum peat moss, 10% perlite and vermiculite; Lambert, Québec, Canada) were used as control (C). Lignite-derived solid humic substances (Novihum Technologies, Salinas, CA, USA), with a composition of 32% humic acid, 3% fulvic acid, and 24% humin, were mixed with the control growing media as an amendment treatment (HS) at the rate of 1% by volume (v/v) basis. The basic physical and chemical properties of the commercial media and humic substances were measured and are shown in Tables 1 and 2.

Table 1. Basic physical properties of the commercial media (CM) and humic substances (HS).

	Size Distribution (%)						TP [1]	AS [2]	CWHC [3]	BD [4]
	<0.25 mm	0.25–0.50	0.50–1.00	1.00–2.00	2.00–2.80	>2.80	(%)	(%)	(%)	(g/cm^3)
CM	0.2	1.1	20.0	55.3	4.9	18.5	58.6	2.9	55.7	0.07
HS	23.4	28.4	29.0	18.0	1.1	0.1	65.8	5.3	60.5	0.61

[1] TP: total porosity; [2] AS: air space; [3] CWHC: container water holding capacity; [4] BD: bulk density.

Table 2. Basic chemical properties of the commercial media (CM), humic substances (HS), and field soil (FS).

	pH	EC [1]	OC [2]	Total N	P	K	Ca	Mg	S	Na	Fe	Zn	Mn	Cu
		(dS/m)	(%)	(%)					(mg/kg)					
CM	5.5	0.08	41.5	0.63	1.85	7.52	938	123	14.82	25.70	19.43	4.56	10.44	0.72
HS	7.4	0.44	65.7	5.48	2.90	56.22	451	334	46.52	452.18	4.18	0.04	4.59	0.04
FS	8.0	0.29	2.0	N/A [3]	47.03	801.68	11355	206	19.05	4.13	2.99	0.78	15.29	0.73

[1] EC: electrical conductivity; [2] OC: organic carbon; [3] N/A: not available.

2.2. Growth Environments and Stress Treatments

After sowing seeds, all trays received irrigation to about 60% water holding capacity and were incubated in a growth chamber (PGR15, Conviron, Winnipeg, Canada) in darkness at 25 °C for 48 h. All trays were then transferred to a greenhouse with an overhead motorized spraying boom system (total length 7.1 m with two long arms at sides and operating orbit at center; each arm has 3.2 m length with 13 sprinkler units) for delivering uniform irrigation and fertilization. Environmental conditions (temperature and humidity) inside the greenhouse were controlled by a Wadsworth control system (Arvada, CO, USA) and hourly monitored by a weather station WatchDog (Spectrum Technologies Inc., Aurora, IL, USA) (Figure 1). After six weeks of growth, seedlings were transplanted in a field with raised beds at the Texas A&M AgriLife Research and Extension Centers in Uvalde, Texas (29.21° N, 99.79° W) with a clay soil type (41% clay, 31% sand, 28% silt) (Table 2). The field was prepared using ridge tillage. Planting configuration–number of rows per bed, distance between plants and beds for peppers were double-row, 0.3 m and 1.8 m; for tomatoes were single-row, 0.46 m and 1.8 m; for watermelons were single-row, 0.6 m and 2.4 m; for lettuces were double-row, 0.25 m and 0.9 m, respectively. Drip irrigation with emitter rate at 0.87 L per hour and emitter spacing at 30 cm (Netafim, Fresno, CA, USA) was installed at 10–15 cm below the soil surface in the center bed and was used for all vegetables tested in this experiment. White plastic mulch was used for pepper and tomato, black for watermelon, and bare soil for lettuce.

During the field growing period, all transplants were subjected to two environmental treatment factors: heat and drought stresses. Heat stress was naturally imposed by growing seedlings during a hot season as compared with no stress with seedlings grown during a cool season. The average field maximum, mean, and minimum temperatures for the cool season were 30.4 °C, 24.1 °C, and 18.7 °C and for the hot season were 35.2 °C, 28.6 °C, and 22.9 °C, respectively (Figure 1). Drought stress was imposed by applying deficit irrigation using an evapotranspiration (ET)-based irrigation scheduling (deficit 50% ET vs. full irrigation at 100% ET). The ET crop water requirement was calculated based on the specific crop coefficients (Kc), flow rate of the drip tape, mulch covering, and precipitation [20]. The differential irrigation treatments started 10 days after transplanting, while fertilization was kept the same among treatments and other standard management practices (weeding, pest and disease control, pruning, trellis, etc.) were followed during the growing period. Within each cultivar/crop/growing season (cool vs. hot) after transplanting, the field layout was a split-plot design with four blocks–irrigation level (50% ET vs. 100% ET) as the whole-plot factor and amendment treated transplants (control vs. HS) as the split-plot factor.

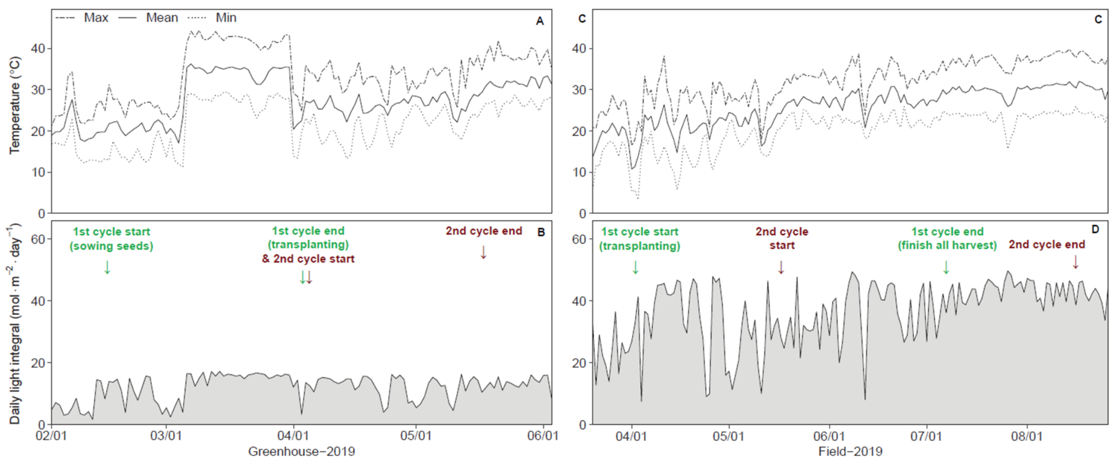

Figure 1. Temperature, daily light integral, and growing cycles (cool and hot seasons indicated by arrows) of greenhouse (**A**,**B**) and field (**C**,**D**) from 1 February, 2019 to 30 August, 2019.

2.3. Seedling and Transplant Quality Evaluation and Yield Performance

Seedling emergence was counted for all crops within 1 to 2 weeks after seeding. During each growing cycle, 4 plants per cultivar/crop from each treatment (C and HS) were randomly sampled from the growing trays at 4 weeks after seeding (WAS), 5 WAS, 6 WAS, and 10 days after transplanting (DAT) for seedling (plants defined as before transplanting) and transplant (after transplanting) evaluation. Plants were removed from the trays and separated by leaf, stem, and root components. The whole roots were carefully washed, scanned using an EPSON V700 scanner (Epson, Long Beach, CA, USA), and then root length (RL), root surface area (RSA), and root average diameter (RAD) were obtained by using WinRHIZO software (Regent Instruments, Québec, Canada). After taking pictures of all leaves with a 1 cm^2 square scale, ImageJ [21] was used for measuring leaf area (LA). Leaf, stem, and root dry weight (LDW, SDW, RDW) were measured after oven drying at 75 °C for 2 days. Leaf area ratio (LAR, ratio of leaf area to plant total dry weight), root/shoot ratio (R:S, ratio of root to shoot dry weight), specific root length (SRL, ratio of root length to root dry mass) were then calculated. Relative growth rate (RGR, calculated based on leaf, stem, root, and total plant) and net assimilation rate (NAR, the increases in plant dry mass per unit leaf area and time) were also calculated based on the following equations. For convenience, all abbreviations are listed in Table S1.

$$RGR = ((\ln(DW_{time1}) - \ln(DW_{time2}))/(time1 - time2) \quad (1)$$

$$NAR = ((DW_{time1} - DW_{time2}) \times ((\ln(LA_{time1}) - \ln(LA_{time2})))/((LA_{time1} - LA_{time2}) \times (time1 - time2)) \quad (2)$$

All plants were kept growing in the field under the two treatment factors (cool vs. hot season; well-watered vs. deficit irrigation) until final harvest. Pepper, tomato, and watermelon were harvested at different times during the growing season, while lettuce was once-over harvested when the majority of heads reached maturity. The total yield was calculated and the average fruit weight (AFW) for pepper, tomato, and watermelon and average head weight for lettuce were calculated based on the total number of fruits (or heads) harvested.

2.4. Statistical Analysis

Seedling and transplant evaluation parameters were analyzed considering media-amendment (Control vs. HS) as the main factor with 8 replications from both growing seasons; while yield performance was analyzed following the split-plot design. R [22] was used for performing ANOVA and means were separated by the least significant difference (LSD) test at 4 levels: $P \leq 0.1$, 0.05, 0.01, 0.001.

3. Results

Based on the two cycles of growth, there were no significant differences of seedling emergence percentage between control and humic substances (HS)-treated growing media (Table S2). Pre- and post-transplanting time-course growth data for each crop species and cultivars are presented in separate graphs.

3.1. Pepper

Compared with untreated control plants (Figure 2), bell pepper (cv. Hunter) grown in HS-added substrate had significantly higher LDW before transplanting and RDW after transplanting ($P < 0.001$). Although there were no significant differences in SDW, HS-treated seedlings had a faster stem RGR than control before transplanting ($P < 0.05$). Lower root-to-shoot ratio (R:S) was observed in HS-treated plants before transplanting compared with control, but the difference disappeared after transplanting, which may be caused by the increases in root growth (RDW). There were no significant differences in NAR, SRL, and RAD. Regarding yield responses, HS-treated transplants had higher yield compared with control under water stress (50% ET) in both cool ($P < 0.1$) and hot seasons ($P < 0.05$), but no differences were found in well-watered treatment (100% ET). HS amendments decreased bell pepper AFW under well-watered treatment in hot season ($P < 0.1$). In bell pepper, the highest RGR increase between 5 and 6 weeks of growth was mostly due to stem rather than root or leaf growth.

Similar RGR trends were observed in HS-treated jalapeño pepper (cv. Jalafuego), which in addition showed a significantly faster RGR in roots after transplanting ($P < 0.05$). Lower R:S were also observed in HS-treated plants before transplanting, but R:S significantly increased after transplanting as compared with control ($P < 0.1$), which could be explained by the significant enhancement of root growth traits (RDW, RL, RSA, $P < 0.05$). There were no significant differences in NAR, SRL, yield, and average fruit weight (AFW) due to the HS application. In field production, both bell and jalapeño peppers had lower yield and AFW in hot temperature as compared with the cool season ($P < 0.001$), and in water stress compared with no stress ($P < 0.01$) (Figure 2 and Table 3).

3.2. Tomato

Compared with untreated control plants (Figure 3), HS-treated round tomato (cv. HM1823) had significantly higher LDW, SDW, and RDW before and after transplanting ($P < 0.05$). RGR was also higher, especially in stem; however, NAR was lower during early growth (4–5 WAS, $P < 0.05$), but these differences were reversed 10 DAT. Similarly, R:S was lower before transplanting but higher after transplanting ($P < 0.1$). In terms of root traits, RL and RSA were significantly higher, especially after transplanting ($P < 0.001$), RAD was also higher ($P < 0.1$), but SRL was lower. HS-treated transplants had higher yield compared with control under no stress conditions (100% ET and cool season) ($P < 0.1$).

In cherry tomato (cv. Sakura), HS had early beneficial effects on leaf and root growth even at 4WAS, with additional faster root RGR after transplanting and higher RL, RSA, RAD during seedling growth and transplant periods than control ($P < 0.01$). Yield was significantly higher for HS than the control under well-watered conditions ($P < 0.001$). For both round and cherry tomatoes, deficit irrigation treatment (50% ET) had significant negative effects on yield during the cool season but not during the hot season ($P < 0.001$). Under heat stress, plants exhibited a dramatic decreased in tomato yield and AFW, especially on cherry tomato ($P < 0.001$) (Figure 3 and Table 3).

3.3. Watermelon

Compared with untreated control plants (Figure 4), HS-treated diploid seeded watermelon (cv. Estrella) had lower leaf and root RGR between 4 and 5 WAS, but higher root biomass ($P < 0.1$) and RGR ($P < 0.05$) were observed 10 DAT. R:S was lower before transplanting, but these differences disappeared after transplanting. Similar trends were observed for NAR. HS-treated transplants had higher SRL but lower RAD at 6 WAS, and higher RL and RSA ($P < 0.05$) at 10 DAT. Although not significant, HS-treated plants had a numerical yield increase of diploid watermelon in the cool season regardless of irrigation treatments.

Triploid seedless watermelon (cv. Fascination) had different root and shoot growth responses as compared with Estrella. HS-treated transplants had faster leaf and stem RGR than control ($P < 0.1$) during the field establishment period (up to 10 DAT), biomass accumulation was accordingly increased although not significant. Before transplanting, RL and RSA were not affected by HS application, but they significantly increased at 10 DAT ($P < 0.05$). These root responses were consistent with those found in the diploid watermelon. SRL was higher for HS plants compared with control at 6 WAS, but similar after transplanting. During the cool season, HS-treated plants had a numerically increased yield under both irrigation rates. HS also increased yield of triploid watermelon in the hot season, particularly for the well-watered treatment ($P < 0.05$). Comparing both stresses, heat stress (high temperature) had more negative dominant effects on yield and AFW of both diploid and triploid watermelons ($P < 0.001$) as compared with water stress (Figure 4 and Table 3).

3.4. Lettuce

Compared with untreated control plants (Figure 5), HS-treated romaine lettuce (cv. Sparx) had significantly higher LDW ($P < 0.001$), faster leaf RGR ($P < 0.05$), but lower RDW before transplanting; however, RDW and root RGR were significantly higher after transplanting ($P < 0.05$). R:S was significantly lower during seedling development and after transplanting. For root traits, RL and RSA were not affected by HS, but SRL was higher before but lower after transplanting, and the reverse responses were measured for RAD. HS-treated romaine lettuce had a significant increase in yield and average head weight (AHW) in the hot season regardless of irrigation treatments ($P < 0.05$).

Butterhead lettuce (cv. Buttercrunch) had similar results as Sparx, with additional significantly lower NAR ($P < 0.05$) and no differences in final yield (though numerically lower during the cool season) comparing HS- with control-treated plants. Heat stress (hot season) had significant negative effects on yield of both romaine and butterhead lettuce types ($P < 0.001$), while the impacts from irrigation treatments were relatively low (Figure 5 and Table 3).

Table 3. ANOVA of total yield, average fruit weight (AFW) as influenced by amendments (A) and irrigation (IR) treatments during the two growing seasons (S).

ANOVA	Pepper		Tomato		Watermelon		Lettuce	
	Bell	Jalapeño	Round	Cherry	Diploid	Triploid	Romaine	Butterhead
				Yield				
S	***	***	***	***	***	***	***	***
IR	**	***	***	***	NS	NS	NS	NS
A	NS	NS	†	*	*	*	**	NS
S × IR	NS	*	***	***	NS	NS	NS	NS
S × A	NS	NS	†	*	NS	NS	NS	*
IR × A	*	NS	*	***	NS	NS	NS	NS
S × IR × A	NS	NS	†	***	NS	NS	NS	NS
				AFW				
S	***	***	*	***	***	***	***	***
IR	NS	**	*	*	NS	NS	NS	NS
A	NS	NS	†	NS	NS	NS	**	NS
S × IR	NS	†	NS	NS	NS	NS	NS	NS
S × A	NS	NS	NS	NS	NS	NS	NS	*
IR × A	NS	NS	NS	NS	NS	NS	NS	NS
S × IR × A	NS	NS	NS	NS	NS	NS	NS	NS

†, *, **, *** show significant difference at $P \leq 0.1$, 0.05, 0.01, and 0.001, respectively; NS, not significant at $P \leq 0.1$.

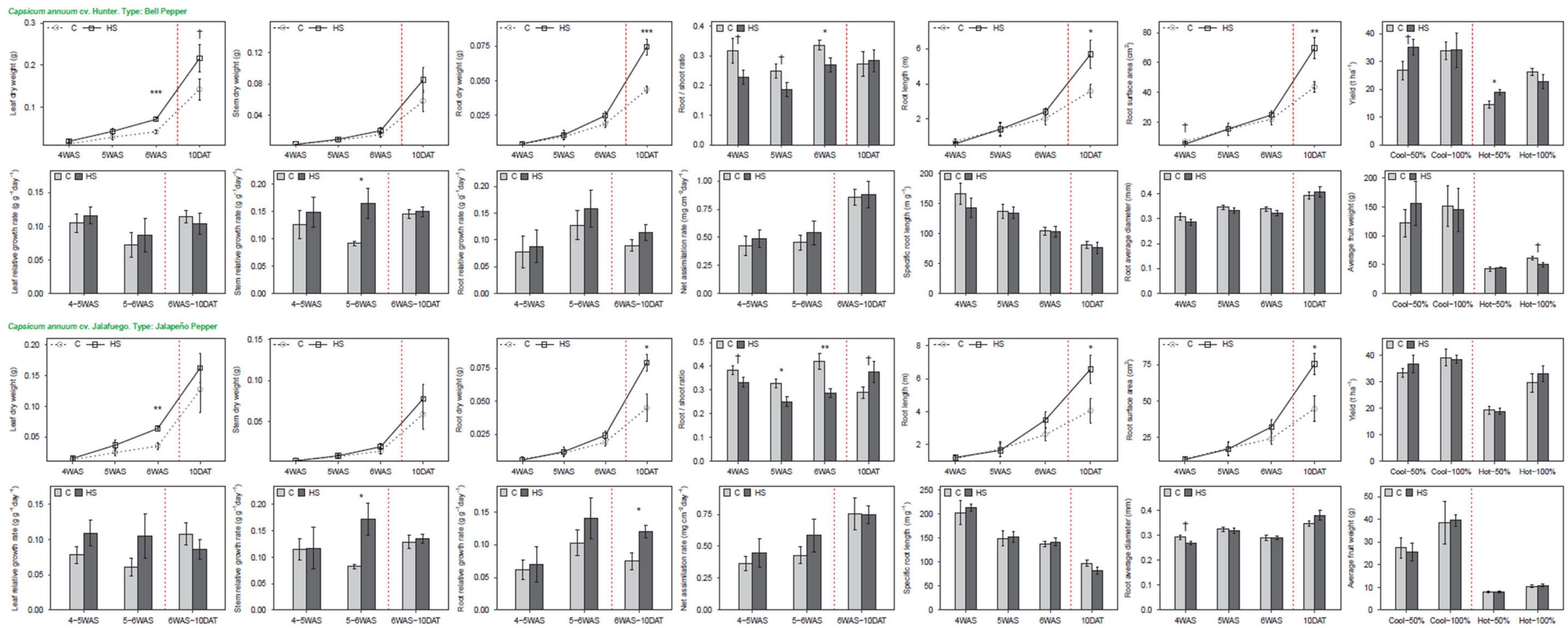

Figure 2. Pepper seedling and transplant quality traits as affected by media amendments, yield traits as affected by amendments and irrigation during the two growing seasons. †, *, **, *** show significant difference comparing HS to control (C) at $P \leq 0.1$, 0.05, 0.01, and 0.001, respectively.

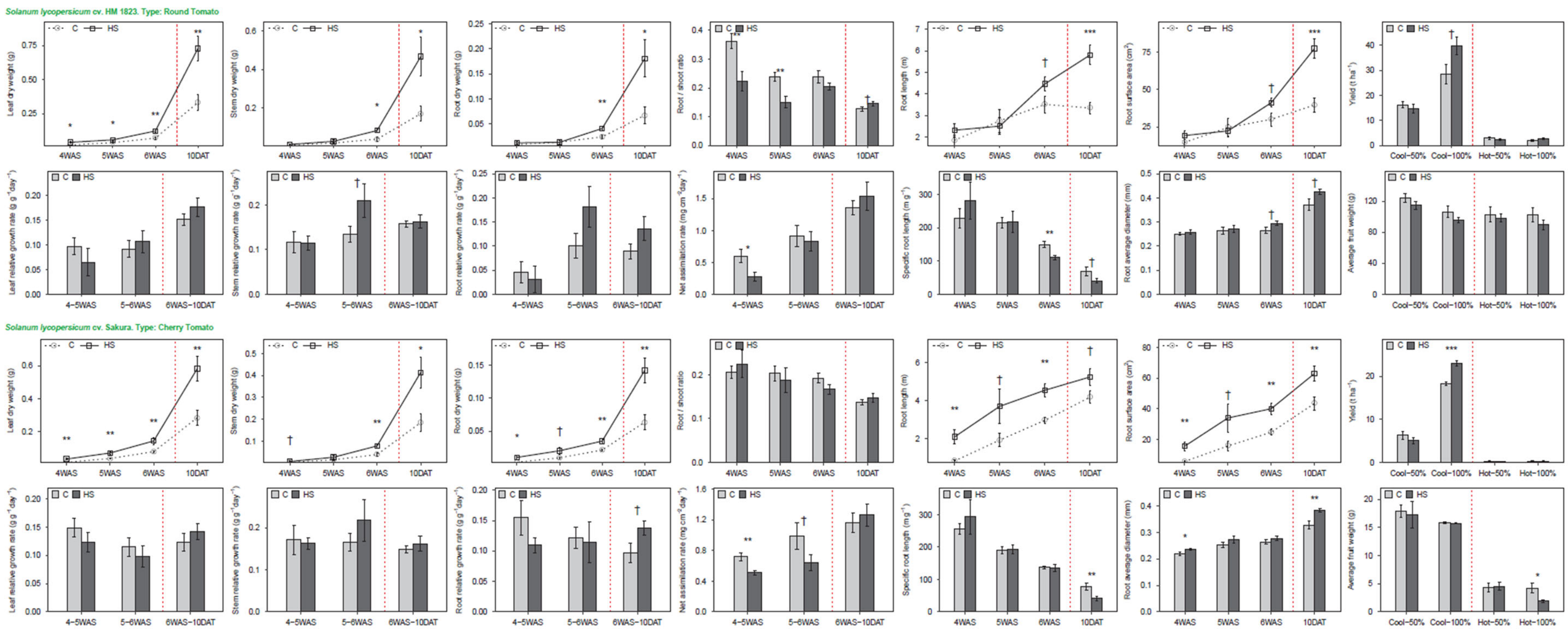

Figure 3. Tomato seedling and transplant quality traits as affected by media amendments, yield traits as affected by amendments and irrigation during the two growing seasons. †, *, **, *** show significant difference comparing HS to control (C) at $P \leq 0.1$, 0.05, 0.01, and 0.001, respectively.

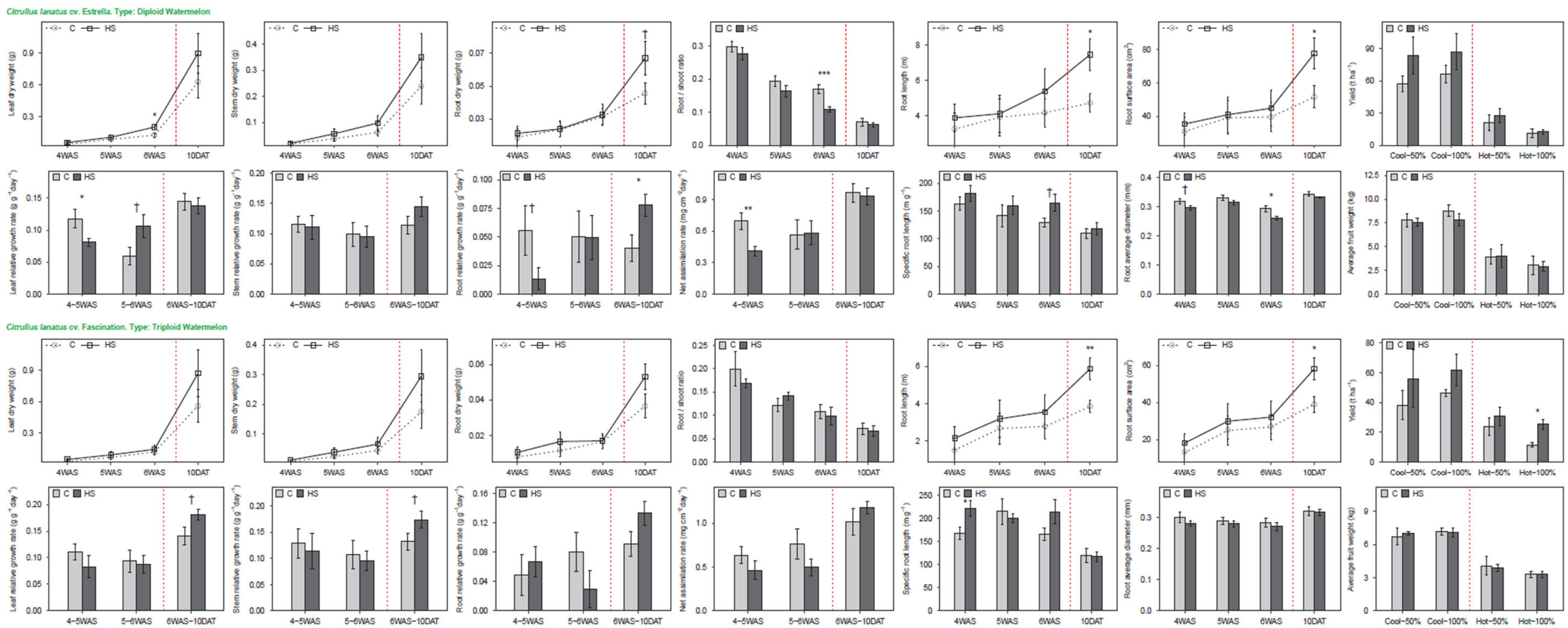

Figure 4. Watermelon seedling and transplant quality traits as affected by media amendments, yield traits as affected by amendments and irrigation during the two growing seasons. †, *, **, *** show significant difference comparing HS to control (C) at $P \leq 0.1$, 0.05, 0.01, and 0.001, respectively.

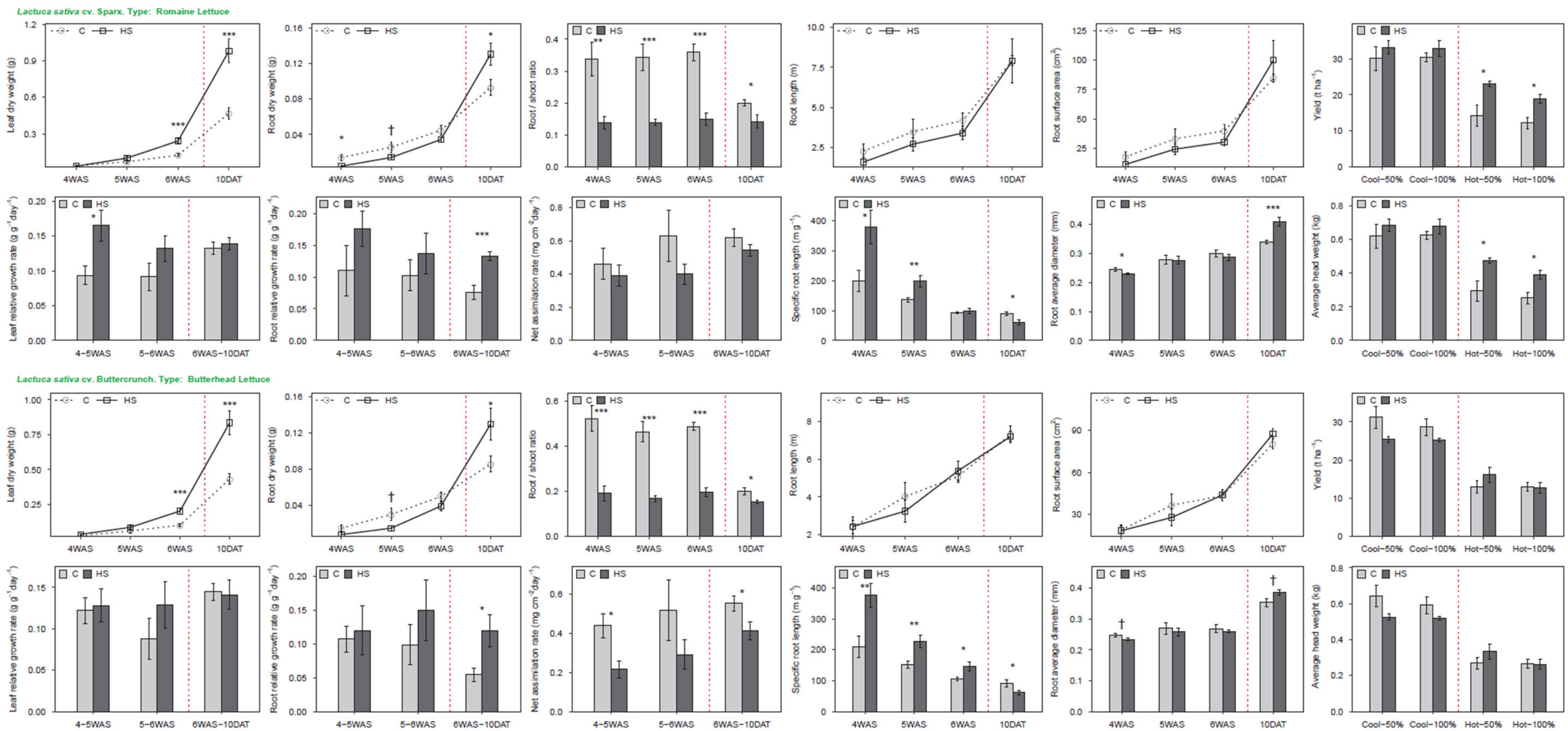

Figure 5. Lettuce seedling and transplant quality traits as affected by media amendments, yield traits as affected by amendments and irrigation during the two growing seasons. †, *, **, *** show significant difference comparing HS to control (C) at $P \leq 0.1$, 0.05, 0.01, and 0.001, respectively.

4. Discussion

Adding solid organic amendments such as compost and vermicompost (derived from organic waste) in growing media have shown benefits in transplant growth [12,23]. However, it is recognized that these amendments that contain high soluble salts could adversely affect germination by lowering the osmotic potential of the water in the media [24]. Since seed germination and seedling emergence are rapid and powerful ways to test potential substrate phytotoxicity [25], they should be fully examined before evaluating seedling or transplant quality. In our study, there were no significant differences in germination percentage and seedling emergence between control and humic substances (HS)-treated growing media, indicating that 1% (v/v) HS was safe and not phytotoxic on seeds tested (Table S2). The overall effects of HS amendments on leaf and root traits, RGR, NAR, yield, and average fruit weight are summarized in Table S3. We found that due to the HS application, leaf, stem, and root biomass accumulation were significantly improved, which could have resulted from higher carbon input from leaves and nutrient absorption from the root.

The HS used in this study were obtained by using the ammonoxidation procedure (lignite reacting with oxygen in aqueous ammonia) and resulted in a product with lower hydrophobicity (mainly caused by reduced aromatic compounds) and higher bioactivity than naturally slow-generated HS from lignite [26]. In addition, solid HS contain humin, which has less hydrophilic carboxyl and hydroxyl groups but higher hydrophobic alkyl groups and ash contents [18]. Raw materials also decide HS properties: lignite-derived HS are composed of highly oxidized sulfur-containing molecules and aromatic and aliphatic groups, which can give the products a higher hydrophobic protection than other raw materials (e.g., peat, compost, sludge, leonardite). This makes them more stable in terms of their existence (lifespan) in the soil solutions, having slowly beneficial effects [27,28]. This HS product contained higher N, K, Mg, and Na contents than commercial media, however, by adding HS with 1% v/v, the nutrient differences compared with control (solely commercial media) were minimized. The similar early growth performance (4 or 5 WAS) also indicated that there were no initial nutrient differences between control and HS-treated trays. During the seedling growth period, the fertilization amount applied for both control and HS trays were exactly the same and sufficient for seedling growth, thus the beneficial effects from HS were probably not related with nutrients. We found the increased seedling biomass in HS-treated trays mainly occurred at a later seedling growth stage (6 WAS) and during early field establishment, with prominent effects on root development. This could indicate the positive results from HS were mainly due to their biostimulation (auxin-like) effects on enhancing plant root development and nutrient acquisition [13], which occurred slowly due to the solid HS product. Since transplant quality was the main focus, below we explain in detail the effects of HS on the specific transplant growth parameters.

As a growth speed index, relative growth rate (RGR) can be affected by internal (species, seed mass, growth cycle) and external physical and environmental factors (pot volume, light, nutrients, and temperature) [29]. Based on the variability, RGR could be used as an indicator for separating functional strategies of plant growth: faster RGR indicates more competition for obtaining growing resources, slower RGR indicates more stress tolerance [30]. Variation of RGR could be predicted by NAR (representing the balance of photosynthetic and respiration rates) or LAR (representing the deployed efficiency of photosynthetic resources) [31–33]. In our study, a significantly positive correlation between RGR and NAR was found only in fruit-based vegetables (pepper, tomato, watermelon), while a significantly positive correlation between RGR and LAR was detected only in the leaf-based vegetable (lettuce) (Figure 6). This could indicate that the growth rate of fruit-based vegetables was determined by both photosynthesis and respiration, while leaf-based vegetables were mainly affected by their photosynthetic resources. In addition, HS-treated transplants had an overall higher RGR (especially root) than control transplants regardless of crop species, which showed a stronger recovery and adaptability (less transplant shock) during the field establishment period, and also indicated a higher nutrient uptake since nutrient absorption correlated with growth rate [34].

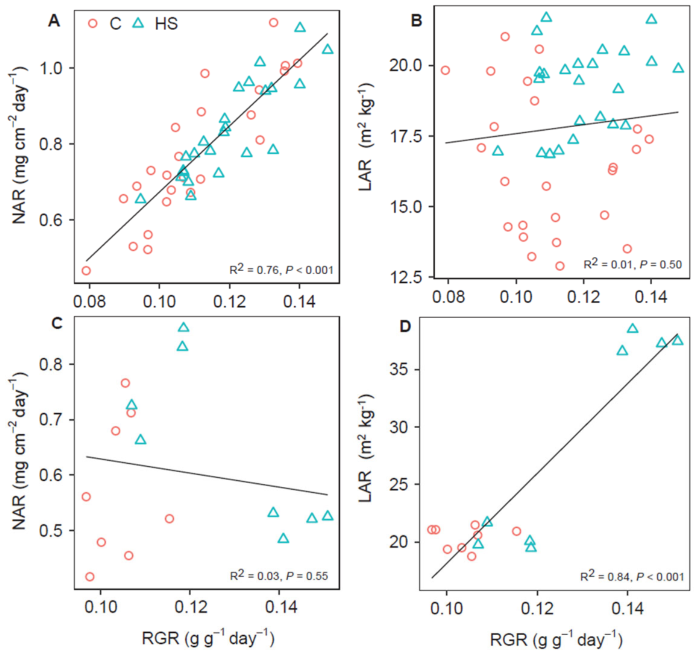

Figure 6. Linear regression plotted for net assimilation rate (NAR) and leaf area ratio (LAR) against relative growth rate (RGR) of (**A**,**B**) fruit-based vegetables (pepper, tomato, watermelon) and (**C**,**D**) leaf-based vegetable (lettuce).

Root-to-shoot ratio (R:S) is an important indicator for the allocation of plant organs against limited growing resources. In general, suitable environments rich in nutrients improve shoot (leaf and stem) growth, while poor environments with insufficient nutrients improve root relative to shoot growth. In seedling production, it is well accepted that R:S is found lower with higher substrate nutrient supply, particularly nitrogen [35]. In our study, lower R:S found in HS-treated seedlings before transplanting indicated a rich nutrient environment possibly due to the nutrient retention ability from HS. Although the boundaries of the optimum R:S are difficult to define, transplants with higher R:S are often considered to have better growth capacity and quicker establishment after transplanting [36]. HS-treated plants (except lettuce) had higher R:S than control plants after transplanting, which could explain the improvement in field establishment and yield performance.

Specific root length (SRL) is a trait that identifies the economic return (represented by root length, RL) from the cost (represented by root dry weight, RDW). The increase of SRL is often associated with nutrient limitation or dry environments [37]. However, an increase in nutrients could also lead to a higher SRL, especially when supplied in a localized nutrient patch, but the proliferation of fine root length was not accompanied by more allocation to root biomass [38]; meanwhile, this situation is species-specific [39]. SRL is strongly dependent on fine roots; with decreased RAD, SRL increased [40]. In our study, compared HS- with control-treated seedlings, RAD was lower before but higher after transplanting; in contrast, SRL changed from higher to lower (except for tomato cultivars). In seedling production before transplanting, nutrients provided in the trays are localized, thus the higher SRL was probably due to a better productive environment with HS, but after transplanting in the field, soil nutrient supply was not as localized as in trays, with lower SRL from HS-treated plants regardless of crops, indicating a less initial stress than control during the transplant shock period. In addition, a significantly increased RDW demonstrated that HS improved plant capacity for rapid root regeneration and growth for larger structural roots during field establishment.

Temperature and irrigation play important roles in vegetable production as they modulate vegetative and reproductive development. In general, flowers are the most temperature-sensitive organs, with high temperature (heat stress) decreasing pollen viability and fruit set, disturbing root functional water and nutrient uptakes, as well as causing abnormal development of shoot tip [41]. Drought stress will impair cell division and leaf area expansion, decrease leaf photosynthetic rate, and delay the conversion of vegetative to reproductive stage [42]. In our study, both heat and water stress decreased crop yield and average fruit weight (size), with heat stress having more significant effects than drought stress. Although within each crop, cultivars representing unique types had different responses, we found that stronger transplant quality due to HS application could ameliorate the adverse effects caused by the abiotic stresses, which led to a higher yield compared with control. These included: bell pepper under drought and heat stresses; round and cherry tomatoes under optimized environment (no stress); triploid watermelon under heat stress without irrigation limitation; romaine lettuce in heat stress regardless of irrigation rates.

In order to better understand the general HS effects on all crop cultivars tested and build linkages between measured seedling or transplant quality traits and subsequent yield, heatmaps (Figure 7) were created based on standardized data sets obtained before and after transplanting. Treatments were clustered based on their measured variables, and variables were clustered based on their correlations (closer meant higher positive correlations). We found that either before or after transplanting, HS treatments were clearly distinguished from control in all crops, mainly due to the higher shoot (SDW), root dry weight (RDW), root length (RL), root surface area (RSA), and yield. Yield was highly correlated with shoot growth (SDW) before transplanting, and root growth traits (RL, RDW, RSA) after transplanting, which indicated that during the seedling stage, sufficient nutrients should be kept in the growth media to improve the plant above-ground growth, while after transplanting, management practices aimed at improving root development should be considered. Besides the application of solid HS in this study, the use of other biostimulant substrates (phenols, salicylic acid, humic and fulvic acid, seaweed extracts, protein hydrolases) and microbial inoculants (plant-growth-promoting rhizobacteria and mycorrhizal fungi) have shown to boost root performance [43,44], which can be used for enhancing transplant field establishment and subsequent crop production. Overall, solid HS with shoot and root growth-promoting effects can satisfy the requirements of transplant growth and subsequent yield in both pre- and post-transplanting environments, which makes them suitable and reliable amendments for use in transplant media.

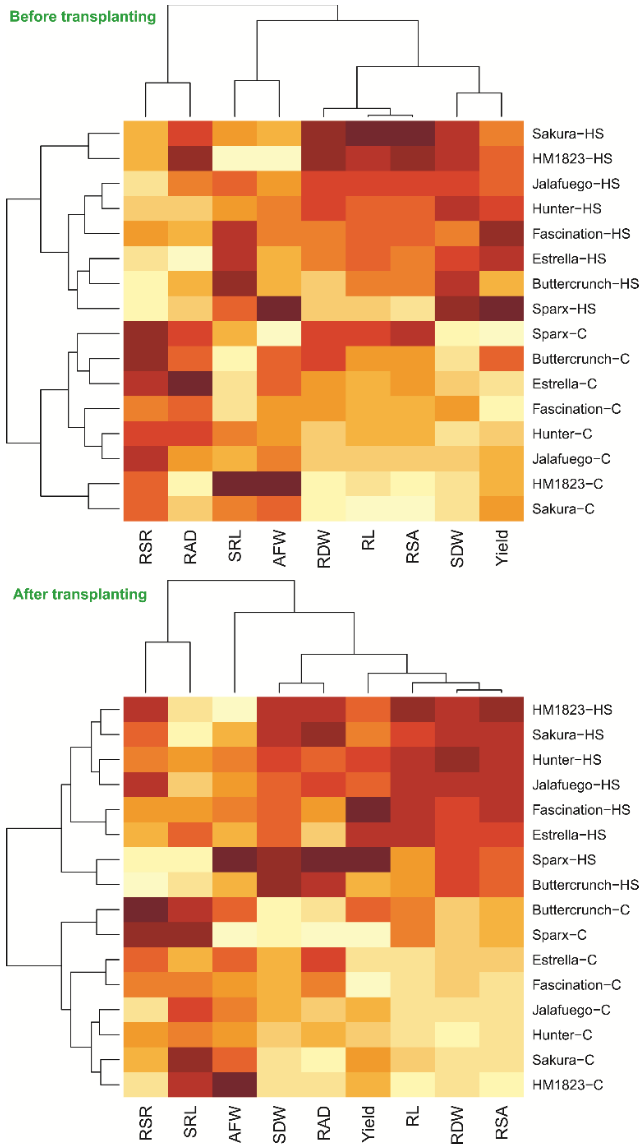

Figure 7. Heatmaps and clustering of the amendment treatments (C and HS) based on the (top) before-transplanting traits and (bottom) after-transplanting traits with the consideration of yield components. Each row represents a crop cultivar with or without HS treatment, and each column represents a measured variable, including shoot dry weight (SDW), root dry weight (RDW), root shoot ratio (RSR), root length (RL), specific root length (SRL), root average diameter (RAD), root surface area (RSA) and average fruit weight (AFW). The expression variable values of the heatmaps follow the red (high)–yellow (low) color scale. All data are standardized and measured variables are clustered based on their correlations.

5. Conclusions

In this study, humic substances (HS) added as a media amendment for growing containerized vegetable transplants were evaluated for their seedling root and shoot growth modulation effects before and after field transplanting. Compared with control, HS:1) improved plant shoot biomass accumulation of pepper, tomato, and lettuce mostly due to faster shoot growth rates, while these effects were not prominent in watermelon; 2) enhanced pepper and watermelon root developmental traits (RDW, RL, RSA) after transplanting due to faster root growth rates, and tomato root development both before and after transplanting, while these effects were not shown in lettuce; 3) decreased net assimilation rate of tomato, watermelon, and lettuce before transplanting but improved after transplanting, while this effect was not significant for pepper; 4) improved leaf area ratio in all four crops; 5) improved specific root length of tomato, watermelon, and lettuce before transplanting but decreased it after transplanting; 6) lowered root-to-shoot ratio of all the crops before transplanting but reversed it after transplanting, except for lettuce. Based on the field performance, we found suitable R:S ranges for high-quality transplants to be as follows: 0.25–0.35 for pepper, 0.15–0.2 for tomato, 0.1 for watermelon, and 0.15–0.2 for lettuce. This study demonstrated that HS differentially modulated root and shoot growth based on crop species: root performances were outstanding in fruit-based crops (pepper, tomato, watermelon), while leaf performances were significantly improved in the leaf-based crop (lettuce). Overall, imposed heat and drought stresses had significantly negative effects on crop yield and average fruit weight, but HS-treated plants showed more improved stress tolerance than control plants by mitigating the yield loss. This study showed the potential application of solid humic substances as biostimulants for enhancing transplant quality and crop performance in four economically important vegetable species (tomato, pepper, watermelon, and lettuce).

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Figure S1: Vegetable crops and cultivars used in this study, Table S1: Abbreviations and their full names used in this study, Table S2: ANOVA and means comparison of germination percentage as affected by amendments (A), Table S3: Summary of HS effects on transplant quality traits (pre- and post-transplanting) and yield components (cool vs. hot seasons, low vs. high irrigation rates) compared to control (higher or lower at significant level $P \leq 0.1$).

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