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"Diversified applications of supplemental LED light for greenhouse tomato production in the Mediterranean context"

Presentata da: Elisa Appolloni

Coordinatore Dottorato
Massimiliano Petracci

Supervisore
Francesco Orsini

Supervisore
Xavier Gabarrell Durany

Co-Supervisore
Giorgio Prosdocimi Gianquinto

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SUMMARY

Background

The problems faced by today agriculture are well known. These include limited cultivation resources, such as available fertile soil and water; climate change, with alternation of drought periods and massive rainfall events; increasing world population and its concentration in urban areas; to name some. In this context, the need to approach an innovative agriculture characterized by strong technological input is of fundamental importance. A technological upgrade may help not only to ensure a major and controlled production with the least amount of cultivation inputs, but also to lead to the creation of more sustainable and resilient food systems in which rural and urban production, also passing through a building integration (e.g., rooftop greenhouses), can be merged to foster local production and consumption with consequent economic, social and environmental benefits.

The application of high-tech greenhouses characterized by climate control systems, soilless cultivation for the optimization of water and nutrient resources, and the use of supplemental artificial light under conditions of limited solar radiation, have already demonstrated an effective capacity to ensure increased production, especially for tomato. In high latitude countries, these systems have become the norm, reaching yields of about 60 kg m^{-2} of tomato in the Netherlands, compared to 28 kg m^{-2} in Almeria, Spain. Indeed, the Mediterranean greenhouse farming context continues to apply mainly low-tech cropping systems, given by passive climate control, soil cultivation and suboptimal covers for internal light condition, which can guarantee limited initial investment costs, but affecting productive capacity and efficiency of management costs in the long term.

Among the various aspects to be investigated for a technological and productive upgrade of the Mediterranean area, the application of supplementary LED interlighting still shows limited interest. In fact, the better solar radiation distribution over the year in confront to the northern area countries might suggest an unnecessary application of this technology in the southernmost contexts. However, high-density tomato cultivation with intensive high-wire systems could lead to mutual shading and consequent reduction in photosynthesis and yield, even in case of appreciable amounts of external solar radiation. Applications of interest could also involve off-season production or application in Building-Integrated Agriculture (BIA) such rooftop greenhouses, where municipal regulations for structure and fire safety could limit the incoming radiation in the growing area. Alongside these aspects, additional LED light could also affect some morpho-physological aspects, with consequences on nutraceutical properties, post-harvest quality, and seedlings production.

Objective

The aim of this research was to investigate diversified applications of supplemental LED interlighting for greenhouse tomato production (*Solanum lycopersicum*), with a specific focus on the Mediterranean countries. Accordingly, the targeted research questions were:

- **RQ1**: What is the current state of tomato cultivation with additional LED light and what aspects still need to be explored? (**Chapter 1** and **2**).

- **RQ2**: What is the potential of supplemental LED light to reduce structural shadings and limited transmission of solar radiation in case of tomato cultivation in Integrated Rooftop Greenhouses (i-RTGs)? (**Chapter 3**).
- **RQ3**: Could supplemental LED light during cultivation affect post-harvest quality of tomato? (**Chapter 4**).
- **RQ4**: Beside fruit production, could the application of supplemental LED light interest other commercial areas such as tomato seedlings production? (**Chapter 5**).
- **RQ5**: Could the use of additional LED light influence some management aspects of tomato cultivation such as defoliation, whit consequences on qualitative and quantitative aspects of production? (**Chapter 6**).

Materials and Methods

The method used for research development involved two phases: a first phase of defining the state of the art of the research topic and possible aspects to be implemented (**RQ1**), and a second phase of experimental application basing on the previous observations (**RQ2**, **3**, **4** and **5**). The first phase was carried out with a systematic and meta-analytic method, and consisted in: the evaluation of the research outputs of the available literature on the topic of supplemental LED light for tomato cultivation (**Chapter 1**), and the analysis of worldwide development of Rooftop Agriculture (RA) as a potential sector of supplemental LED light application (**Chapter 2**). The second phase consisted in the development of four experiments (**Chapter 3**, **4**, **5**, **6**). The first experiment aimed to answer the **RQ2**, applying the following light treatments: Red and Blue in a 3 ratio (RB), Red and Blue in a 3 ratio + Far-Red (FR) the whole day, and Red and Blue in a 3 ratio + Far-Red at the end-of-day for 30 min (EOD). The light treatments were added to natural sunlight for 16 h d^{-1} (8-00 am) with an intensity around 170 µmol m^{-2} s^{-1}. A control grown under natural light was also considered (CK). The second experiment answered the **RQ3**, applying the same treatments of first experiment to evaluate the effects of supplemental LED light during cultivation on tomato post-harvest quality, after 1 week of storage in the dark at 13°C. The third experiment answered the **RQ4**, using the light treatments of previous experiments to evaluate morpho-physiological response of tomato seedlings. Finally, the fourth experiment, that answered the **RQ5**, considered the combination of two defoliation regimes, namely leaf removal before harvesting (R) or non-removal (NR), with RB or CK treatments.

Results

RQ1: The meta-analytical evaluation of the application of supplementary LED light for tomato cultivation showed an effective ability to increase the yield (+40%), soluble solid (+6%) and ascorbic acid (+11%) contents, leaf chlorophyll content (+31%), photosynthetic capacity (+50%) and leaf area (+9%). The analysis also revealed a limited application of the technology in the Mediterranean %

worldwide development of RA showed a growth of the sector, with potentially interesting scope applications of additional LED light to improve rooftop greenhouse production (**Chapter 1** and **2**).

RQ2: The application of supplemental LED light in an i-RTG showed the potential to overcome light limitations due to structural shadings and low transmissivity of fireproof covering materials independently of spectrum quality. In particular, LED treated plants achieved a yield increase by 17% compared to the control grown under natural light (CK), which showed 9.3% lighter and 7.2% fewer fruits (**Chapter 3**).

RQ3: Pre-harvest application of LED supplemental lighting positively affected the post-harvest quality of tomatoes after one week of storage at 13°C. Particularly, RB and FR increased fruit firmness compared to CK. Furthermore, RB fruit maintained a higher content of lycopene and β-carotene after the one week of storage compared to CK (**Chapter 4**).

RQ4: Supplemental LED light showed affect growth indexes and morpho-physiological response of tomato seedlings depending on lighting treatment. In particular, RB and FR treatments resulted in improved plants compactness, contemporarily guaranteeing a good chlorophyll content. Among treatments supplied with artificial lighting, EOD plants presented longer hypocotyls, still maintaining high chlorophyll content. On the other hand, CK plants presented longer hypocotyls, higher leaf area and lower chlorophyll content, also showing higher Specific Leaf Area (SLA) and Leaf Area Ratio (LAR) (**Chapter 5**).

RQ5: The application or not of defoliation seems to have not significant effects on tomato plants yield and other vegetative parameters, independently of combination with supplemental LED light (RB) or natural light alone (CK). However, plants subjected to leaf removal showed a significantly decreased content of soluble solids, also presenting a higher transpiration. The application of RB treatment showed a significant capacity to increase tomato total yield (+118%) as compared to CK during the wintertime (**Chapter 6**).

Conclusions

From the results, it is possible to conclude that the application of supplemental LED light on greenhouse-grown tomato, with a specific focus on the Mediterranean countries, has potential to foster diverse applications. In particular, it can increase production in case of the limited solar radiation in i-RTGs, maintain quality and reduce losses during post-harvest, and showed potential for the sector of seedlings production.

Suggestions for future research

Despite the positive results obtained, some aspects of the application of additional LED light in Southern Europe countries still need to be deepened and improved. In particular, given the current increase of electricity cost, future research should focus on more economically valuable methods of managing supplemental lighting, such as the application of shorter photoperiods or lower intensities, or techniques that can provide energy savings such as the pulsed light. Further investigation should also concern post-harvest quality evaluation, considering longer storage periods and evaluations of tomatoes at earlier stages of development. Moreover, the EOD treatment, which showed a greater elongation of the hypocotyls in tomato seedlings, could be tested on grafting varieties to favor the production of easier manageable and higher quality rootstocks. Finally, the extension of tomato seedlings evaluation until fruiting stage should also be considered to better understand the effects that additional light at the beginning of plant development may have on the distribution of assimilates.

RESUM

Context

Els problemes a què s'enfronta l'agricultura actual són ben coneguts. Entre d'altres s'inclouen els recursos de cultiu limitats, com ara sòls fèrtils i aigua disponible; el canvi climàtic, amb alternança de períodes de sequera i esdeveniments de pluja massiva; l'augment de la població mundial i la seva concentració a les zones urbanes. En aquest context, la necessitat d'apropar-se a una agricultura innovadora caracteritzada per una forta aportació tecnològica és de fonamental importància. Una actualització tecnològica pot ajudar no només a garantir una producció important i controlada amb la menor quantitat d'inputs de cultiu, sinó també a conduir a la creació de sistemes alimentaris més sostenibles i resilients en els quals la producció rural i urbana, passant també per una integració en edifici (per exemple, els hivernacles de les cobertes), es poden fusionar per fomentar la producció i el consum locals amb els consegüents beneficis econòmics, socials i ambientals.

L'ús d'hivernacles d'alta tecnologia caracteritzats per sistemes de control climàtic, cultiu sense sòl per a l'optimització dels recursos hídrics i de nutrients, i l'ús de llum artificial suplementària en condicions de radiació solar limitada, ja han demostrat una capacitat eficaç per garantir un augment de la producció, especialment per tomàquet. Als països de latituds altes, aquests sistemes s'han convertit en la norma, aconseguint uns rendiments d'uns 60 kg m^{-2} de tomàquet als Països Baixos, enfront dels 28 kg m^{-2} d'Almeria, Espanya. Efectivament, el context mediterrani d'hivernacle segueix aplicant principalment sistemes de cultiu de baixa tecnologia, donats pel control climàtic passiu, el cultiu del sòl i les cobertes subòptimes per a condicions de llum interna, que poden garantir uns costos d'inversió inicials limitats, però afectant la capacitat productiva i l'eficiència dels costos de gestió a la llarga.

Entre els diferents aspectes a investigar per a una millora tecnològica i productiva de l'àrea mediterrània, l'aplicació de la il·luminació LED suplementària encara mostra un interès limitat. De fet, la millor distribució de la radiació solar durant l'any enfront dels països de la zona nord podria suggerir una aplicació innecessària d'aquesta tecnologia en els contextos més meridionals. No obstant això, el cultiu de tomàquet d'alta densitat amb sistemes intensius emparrats podria conduir a un ombreig mutu i la consegüent reducció de la fotosíntesi i el rendiment, fins i tot en cas de quantitats apreciables de radiació solar externa. Estratègies d'interès també podrien incloure la producció fora de temporada o l'aplicació a l'agricultura integrada en l'edificació (BIA), com els hivernacles a sobre de terrats, on les regulacions municipals d'estructura i seguretat contra incendis podrien limitar la radiació entrant a la zona de cultiu. Paral·lelament a aquests aspectes, la llum LED addicional també podria afectar alguns aspectes morfofisiològics, amb conseqüències sobre les propietats nutracèutiques, la qualitat postcollita i la producció de plàntules.

Objectiu

L'objectiu d'aquesta investigació va ser investigar aplicacions diversificades de la il·luminació LED suplementària per a la producció de tomàquet d'hivernacle (*Solanum lycopersicum*), amb un focus específic en el context mediterrani. En conseqüència, les preguntes de recerca dirigides van ser:

- **RQ1**: Quin és l'estat actual del cultiu de tomàquet amb llum LED addicional i quins aspectes s'han d'explorar encara? (**Capítol 1** i **2**).

- **RQ2**: Quin és el potencial de la llum LED suplementària per reduir els ombrejats estructurals i la transmissió limitada de la radiació solar en el cas del cultiu de tomàquet en hivernacles integrats a les cobertes (i-RTG)? (**Capítol 3**).

- **RQ3**: La llum LED addicional durant el cultiu podria afectar la qualitat del tomàquet després de la collita? (**Capítol 4**).

- **RQ4**: A més de la producció de fruites, l'aplicació de llum LED suplementària podria interessar altres àrees comercials com la producció de plàntules de tomàquet? (**Capítol 5**).

- **RQ5**: L'ús de llum LED addicional podria influir en alguns aspectes de gestió de la producció de tomàquet, com ara la defoliació, amb conseqüències en els aspectes qualitatius i quantitatius de la producció? (**Capítol 6**).

Materials i mètodes

El mètode utilitzat per al desenvolupament de la recerca va comportar dues fases: una primera fase de definició de l'estat de l'art del tema de recerca i possibles aspectes a implementar (**RQ1**), i una segona fase d'aplicació experimental basada en les observacions anteriors (**RQ2**, **3**, **4** i **5**). La primera fase es va dur a terme amb un mètode sistemàtic i meta-analític, i va consistir en: l'avaluació dels resultats de la investigació de la literatura disponible sobre el tema de la llum LED suplementària per al cultiu de tomàquet (**Capítol 1**), i l'anàlisi del desenvolupament mundial de l’agricultura en coberta (RA) com a sector potencial d'aplicació de llum LED suplementària (**Capítol 2**). La segona fase va consistir en el desenvolupament de quatre experiments (**Capítol 3**, **4**, **5** i **6**). El primer experiment tenia com a objectiu donar resposta a l'**RQ2**, aplicant els següents tractaments de llum: vermell i blau en una proporció de 3 (RB), vermell i blau en una proporció de 3 + Far-Red (FR) durant tot el dia, i vermell i blau en una proporció de 3 + Far-Red al final del dia durant 30 min (EOD). Els tractaments de llum es van afegir a la llum solar natural durant 16 h d^{-1} (8-00 h) amb una intensitat al voltant de 170 µmol m^{-2} s^{-1}. També es va considerar un control cultivat sota llum natural (CK). El segon experiment va respondre a l'**RQ3**, aplicant els mateixos tractaments del primer experiment per avaluar els efectes de la llum LED suplementària durant el cultiu sobre la qualitat postcollita del tomàquet, després d'una setmana d'emmagatzematge a la foscor a 13°C. El tercer experiment va respondre a l'**RQ4**. , utilitzant els tractaments de llum d'experiments anteriors per avaluar la resposta morfo-fisiològica de les plàntules de tomàquet. Finalment, el quart experiment, que va respondre al **RQ5**, on es vaconsiderar la combinació de dos règims de defoliació, és a dir, l'eliminació de fulles abans de la collita (R) o la no eliminació (NR), amb tractaments RB o CK.

Resultats

RQ1: L'avaluació meta-analítica de l'aplicació de llum LED suplementària per al cultiu de tomàquet va mostrar una capacitat efectiva per augmentar el rendiment (+40%), els continguts de sòlids solubles (+6%) i àcid ascòrbic (+11%), contingut de clorofil·la en fulla (+31%), capacitat fotosintètica (+50%) i superfície foliar (+9%). L'anàlisi també va revelar una aplicació limitada de la tecnologia en el context mediterrani, així com una avaluació limitada dels aspectes nutracèutics. A més, l'avaluació del desenvolupament mundial de RA va mostrar un creixement del sector, amb aplicacions addicionals de llum LED potencialment interessants per millorar la producció d'hivernacles a les cobertes (**Capítols 1** i **2**).

RQ2: L'aplicació de llum LED suplementària en un i-RTG va mostrar el potencial per superar les limitacions de la llum a causa dels ombrejats estructurals i la baixa transmissivitat dels materials de recobriment ignífugs independentment de la qualitat de l'espectre. En particular, les plantes tractades amb LED van aconseguir un augment del rendiment d'un 17% en comparació amb el control cultivat amb llum natural (CK), que va mostrar una reducció de pes del 9,3% i un 7,2% menys de fruits (**Capítol 3**).

RQ3: L'aplicació d'il·luminació suplementària LED abans de la collita va afectar positivament la qualitat post-collita dels tomàquets després d'una setmana d'emmagatzematge a 13°C. En particular, RB i FR van augmentar la fermesa de la fruita en comparació amb CK. A més, la fruita RB va mantenir un contingut més elevat de licopè i β-carotè després d'una setmana d'emmagatzematge en comparació amb CK (**Capítol 4**).

RQ4: La llum LED suplementària va mostrar que afecta els índexs de creixement i la resposta morfo-fisiològica de les plàntules de tomàquet en funció del tractament d'il·luminació. En particular, els tractaments RB i FR van donar com a resultat una millora en la compactació de les plantes, garantint al mateix tempsun bon contingut de clorofil·la. Entre els tractaments subministrats amb il·luminació artificial, les plantes EOD presentaven hipocòtils més llargs, mantenint encara un alt contingut de clorofil·la. D'altra banda, les plantes CK presentaven hipocòtils més llargs, major àrea foliar i menor contingut de clorofil·la, mostrant també una àrea foliar específica (SLA) i una proporció d'àrea foliar (LAR) més altes (**Capítol 5**).

RQ5: L'aplicació o la no aplicació de la defoliació sembla no tenir efectes significatius sobre el rendiment de les plantes de tomàquet i altres paràmetres vegetatius, independentment de la combinació amb llum LED suplementària (RB) o llum natural sola (CK). No obstant això, les plantes sotmeses a la retirada de fulles van mostrar un contingut d'acidesa significativament major i un contingut reduït de sòlids solubles, presentant també una transpiració més elevada. L'aplicació del tractament RB va mostrar una capacitat significativament major per augmentar el rendiment de tomàquet (+118%) en comparació amb CK durant l'hivern (**Capítol 6**).

Conclusions

A partir dels resultats, es pot afirmar que l'aplicació de llum LED suplementària al tomàquet cultivat en hivernacle en el context mediterrani té potencial per fomentar diverses millores. En particular, pot augmentar la producció en cas de radiació solar limitada en els i-RTG, millorar la qualitat post-collita i garantir la producció de plàntules de qualitat.

Suggeriments per a futures investigacions

Malgrat els resultats positius obtinguts, encara cal aprofundir i millorar alguns aspectes de l'aplicació de la llum LED addicional en el context mediterrani. En particular, atès l'augment actual del cost de l'electricitat, les investigacions futures haurien de centrar-se en mètodes més econòmics per gestionar la il·luminació suplementària, com l'aplicació de fotoperíodes més curts o intensitats més baixes, o tècniques que puguin proporcionar estalvi energètic com la llum polsada. Adicionalment futures investigaciós també hauria de referir-se a l'avaluació de la qualitat després de la collita, considerant períodes d'emmagatzematge més llargs i avaluacions dels tomàquets en etapes primerenques de desenvolupament. A més, el tractament EOD, que va mostrar un major allargament dels hipocòtils en les plàntules de tomàquet, es va poder provar en varietats d'empelt per afavorir la producció de portaempelts més fàcils de manejar i de més qualitat. Finalment, també s'ha de considerar l'extensió de l'avaluació de les plàntules de tomàquet fins a l'etapa de fructificació per entendre millor els efectes que pot tenir la llum addicional a l'inici del desenvolupament de la planta sobre la distribució dels assimilats.

RESUMEN

Antecedentes

Los problemas a los que se enfrenta la agricultura actual son bien conocidos. Entre ellos, la limitación de los recursos de cultivo, como el suelo fértil y el agua disponibles; el cambio climático, con la alternancia de periodos de sequía y eventos de lluvias masivas; el aumento de la población mundial y su concentración en zonas urbanas; por nombrar algunos. En este contexto, la necesidad de abordar una agricultura innovadora caracterizada por un fuerte aporte tecnológico es de fundamental importancia. Una actualización tecnológica puede ayudar no sólo a garantizar una producción mayor y controlada con la menor cantidad de insumos de cultivo, sino también a la creación de sistemas alimentarios más sostenibles y resilientes en los que la producción rural y urbana, pasando también por una integración en edificio (por ejemplo, invernaderos en azoteas), puedan fusionarse para fomentar la producción y el consumo local con los consiguientes beneficios económicos, sociales y ambientales.

La aplicación de invernaderos de alta tecnología caracterizados por sistemas de control climático, el cultivo sin suelo para la optimización de los recursos de agua y nutrientes, y el uso de luz artificial suplementaria en condiciones de radiación solar limitada, ya han demostrado una capacidad efectiva para garantizar el aumento de la producción, especialmente del tomate. En los países de alta latitud, estos sistemas se han convertido en la norma, alcanzando rendimientos de unos 60 kg m^{-2} de tomate en los Países Bajos, frente a los 28 kg m^{-2} de Almería (España). De hecho, en el contexto de la agricultura mediterránea en invernadero se siguen aplicando principalmente sistemas de cultivo de baja tecnología, dados por el control climático pasivo, el cultivo en suelo y las cubiertas subóptimas para la condición lumínica interna, que pueden garantizar unos costes de inversión iniciales limitados, pero que afectan a la capacidad productiva y a la eficiencia de los costes de gestión a largo plazo.

Entre los diversos aspectos que deben investigarse para una mejora tecnológica y productiva de la zona mediterránea, la aplicación de la interiluminación LED suplementaria sigue mostrando un interés limitado. De hecho, la mejor distribución de la radiación solar a lo largo del año en comparación con los países del área norte podría sugerir una aplicación innecesaria de esta tecnología en los contextos más meridionales. Sin embargo, el cultivo de tomates en alta densidad entutorados en forma vertical podría provocar un sombreado mutuo y la consiguiente reducción de la fotosíntesis y del rendimiento, incluso en caso de cantidades apreciables de radiación solar externa. Las aplicaciones de interés también podrían incluir la producción fuera de temporada o la aplicación en la Agricultura Integrada en Edificios (BIA), como los invernaderos en azoteas, donde las regulaciones municipales para la estructura y la seguridad contra incendios podrían limitar la radiación entrante en la zona de cultivo. Además de estos aspectos, la luz LED adicional también podría afectar a algunos aspectos morfo-fisiológicos, con consecuencias en las propiedades nutracéuticas, la calidad postcosecha y la producción de plántulas.

Objetivo

El objetivo de esta investigación fue investigar aplicaciones diversas de interiluminación LED suplementaria para la producción de tomate en invernadero (*Solanum lycopersicum*), con un enfoque específico en el contexto mediterráneo. En consecuencia, las preguntas de investigación que se plantearon fueron las siguientes:

- **RQ1**: ¿Cuál es el estado actual del cultivo de tomate con luz LED suplementaria y qué aspectos quedan por explorar? (**Capítulos 1** y **2**).
- **RQ2**: ¿Cuál es el potencial de la luz LED suplementaria para reducir los sombreados estructurales y la transmisión limitada de la radiación solar en el caso del cultivo de tomate en invernaderos de cubierta integrados (i-RTG)? (**Capítulo 3**).
- **RQ3**: ¿Podría la luz LED suplementaria aplicada durante el cultivo afectar a la calidad postcosecha del tomate? (**Capítulo 4**).
- **RQ4**: Además de la producción de fruta, ¿podría la aplicación de luz LED suplementaria interesar a otras áreas comerciales como la producción de plántulas de tomate? (**Capítulo 5**).
- **RQ5**: ¿Podría el uso de luz LED adicional influir en algunos aspectos de gestión de la producción de tomate como la defoliación, con consecuencias en los aspectos cualitativos y cuantitativos de la producción? (**Capítulo 6**).

Materiales y métodos

El método utilizado para el desarrollo de la investigación implicó dos fases: una primera fase de definición del estado del arte del tema de investigación y de los posibles aspectos a implementar (**RQ1**), y una segunda fase de aplicación experimental basada en las observaciones previas (**RQ2**, **3**, **4** y **5**). La primera fase se llevó a cabo con un método sistemático y meta-analítico, y consistió en: la evaluación de los resultados de la búsqueda de la literatura disponible sobre el tema de la luz LED suplementaria para el cultivo del tomate (**Capítulo 1**), y el análisis del desarrollo mundial de la Agricultura de Azotea (RA) como un sector potencial de aplicación de la luz LED suplementaria (**Capítulo 2**). La segunda fase consistió en el desarrollo de cuatro experimentos (**Capítulos 3**, **4**, **5** y **6**). El primer experimento tenía como objetivo responder a la **RQ2**, aplicando los siguientes tratamientos de luz: Rojo y Azul en una proporción de 3 (RB), Rojo y Azul en una proporción de 3 + Rojo Lejano (FR) durante todo el día, y Rojo y Azul en una proporción de 3 + Rojo Lejano al final del día durante 30 min (EOD). Los tratamientos de luz se añadieron a la luz solar natural durante 16 h d^{-1} (8-00 am) con una intensidad de alrededor de 170 $\mu mol\ m^{-2}\ s^{-1}$. También se consideró un control cultivado bajo luz natural (CK). El segundo experimento respondió a la **RQ3**, aplicando los mismos tratamientos del primer experimento para evaluar los efectos de la luz LED suplementaria durante el cultivo sobre la calidad postcosecha del tomate, después de 1 semana de almacenamiento en la oscuridad a 13° C. El tercer experimento respondió a la **RQ4**, utilizando los tratamientos de luz de los experimentos anteriores para evaluar la respuesta morfo-fisiológica de las plántulas de tomate. Finalmente, el cuarto experimento, que respondió a la **RQ5**, consideró la combinación de dos regímenes de defoliación, que consistieron en el deshoje antes de la cosecha (R) o el no deshoje (NR), con tratamientos de luz RB o CK.

Resultados

RQ1: La evaluación meta-analítica de la aplicación de luz LED suplementaria para el cultivo de tomate mostró que tiene una capacidad efectiva para aumentar el rendimiento (+40%), los contenidos de sólidos solubles (+6%) y de ácido ascórbico (+11%), el contenido de clorofila de la hoja (+31%), la capacidad fotosintética (+50%) y el área foliar (+9%). El análisis también reveló una aplicación limitada de la tecnología en el contexto mediterráneo, así como una evaluación limitada de los aspectos nutracéuticos. Además, la evaluación del desarrollo mundial de la AR mostró un crecimiento del sector, con aplicaciones de la luz LED suplementaria de alcance potencialmente interesante para mejorar la producción de los invernaderos de techo (**Capítulos 1** y **2**).

RQ2: La aplicación de luz LED suplementaria en un i-RTG mostró su potencial para superar las limitaciones de luz debidas a los sombreados estructurales y a la baja transmisividad de los materiales de cubierta ignífugos, independientemente de la calidad del espectro. En particular, las plantas tratadas con LED lograron un aumento del rendimiento del 17% en comparación con el control cultivado con luz natural (CK), que se mostró un 9.3% más ligero y un 7.2% menos de frutos (**Capítulo 3**).

RQ3: La aplicación de iluminación suplementaria LED antes de la cosecha afectó positivamente a la calidad postcosecha de los tomates tras una semana de almacenamiento a 13°C. En particular, RB y FR aumentaron la firmeza de los frutos en comparación con CK. Además, los frutos RB mantuvieron un mayor contenido de licopeno y β-caroteno después de una semana de almacenamiento en comparación con CK (**Capítulo 4**).

RQ4: La luz LED suplementaria mostró afectar los índices de crecimiento y la respuesta morfo-fisiológica de las plántulas de tomate dependiendo del tratamiento de iluminación. En particular, los tratamientos RB y FR dieron lugar a una mejora de la compactación de las plantas, garantizando al mismo tiempo un buen contenido de clorofila. Entre los tratamientos suministrados con iluminación artificial, las plantas EOD presentaron hipocótilos más largos, manteniendo un alto contenido de clorofila. Por otro lado, las plantas CK presentaron hipocótilos más largos, mayor área foliar y menor contenido de clorofila, mostrando también mayor Área Foliar Específica (SLA) y Relación de Área Foliar (LAR) (**Capítulo 5**).

RQ5: La aplicación o no de la defoliación no parece tener efectos significativos sobre el rendimiento de las plantas de tomate y otros parámetros vegetativos, independientemente de la combinación con luz LED suplementaria (RB) o luz natural sola (CK). Sin embargo, las plantas sometidas al deshoje mostraron un contenido de acidez significativamente mayor y un contenido de sólidos solubles menor, presentando además una mayor transpiración. La aplicación del tratamiento RB mostró una capacidad significativa para aumentar el rendimiento del tomate (+118%) en comparación con CK durante el invierno (**Capítulo 6**).

Conclusiones

A partir de los resultados, se puede afirmar que la aplicación de luz LED suplementaria en el tomate cultivado en invernadero en el contexto mediterráneo tiene potencial para fomentar diversas mejoras. En particular, puede aumentar la producción en caso de que la radiación solar sea limitada en los i-RTG, mejorar la calidad postcosecha y garantizar la producción de plántulas de calidad.

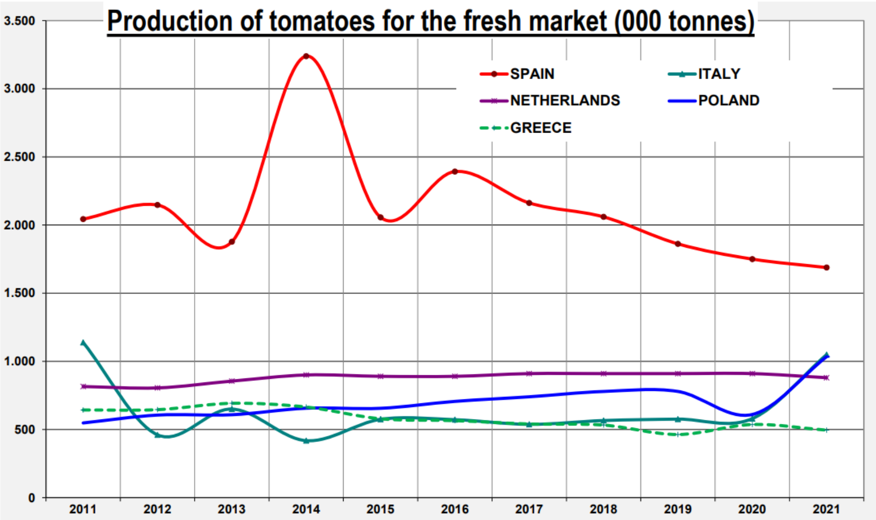
Sugerencias para futuras investigaciones

A pesar de los resultados positivos obtenidos, todavía es necesario profundizar y mejorar algunos aspectos de la aplicación de la luz LED adicional en el contexto mediterráneo. En particular, dado el actual aumento del coste de la electricidad, la investigación futura debería centrarse en métodos más económicos para gestionar la iluminación suplementaria, como la aplicación de fotoperiodos más cortos o intensidades más bajas, o técnicas que puedan proporcionar un ahorro de energía como la luz pulsada. Futuras investigaciónes también debería referirse a la evaluación de la calidad postcosecha, considerando períodos de almacenamiento más largos y evaluaciones de los tomates en etapas más tempranas del desarrollo. Además, el tratamiento EOD, que mostró una mayor elongación de los hipocótilos en las plántulas de tomate, podría ser probado en variedades de injerto para favorecer la producción de portainjertos más manejables y de mayor calidad. Por último, también debería considerarse la prolongación de la evaluación de las plántulas de tomate hasta la fase de fructificación para comprender mejor los efectos que la luz adicional al principio del desarrollo de la planta puede tener en la distribución de los asimilados.

1 INTRODUCTION AND OBJECTIVES

1.1 Tomato production and limits of Mediterranean area

Tomato is one of the most widely produced vegetable crops, along with onion and cucumber (FAOSTAT, 2021). The majors European producers of tomato for fresh consumption include Spain, Italy, Poland, The Netherlands and Greece (EC, 2022) (**Figure 1**). These producers apply cultivation systems with diverse degrees of technology. In particular, The Netherlands are characterized by high-tech cultivation systems, using soilless technique and supplementary LED light, while Mediterranean countries mostly apply low-tech cultivation systems with passive climatic control and on-soil cultivation (Perdossi et al., 2018; Palmitessa et al., 2021a). It has been estimated that less than 10% of protected crops in Italy are grown in soilless systems (Pardossi et al., 2018). However, increasing the application of high-tech soilless cultivation could have interesting implications for sustainability and productivity amelioration even in Mediterranean contexts, as this cultivation technique can help increasing water and nutrient use efficiency (Resh, 2012; AlShrouf, 2017), and determining noticeably larger yield increases per unite of productive surface. For instance, tomato production could move from 28 kg m^{-2} in Almeria, Spain, to 60 kg m^{-2} in The Netherlands (Heuvelink, 2018), applying high-tech and intensive cultivation systems.



Source: EUROSTAT, FAO, WPTC and MS communications; AGRI E.2 elaborations;

Figure 1. Trend of fresh tomato production for the five major European producers (Source: AGRI E.2 elaboration).

Tomato has average light exigencies of around 25 µmol m^{-2} d^{-1} (Palmitessa et al., 2021a). In some cases, ensuring good light levels in the greenhouse environment is not always easy, as in case of productions at high latitudes (e.g., Norway, The Netherlands) with limited yearly light distribution (Kaiser et al., 2019; Paponov et al., 2020), or intensive productions applying high density high-wire systems, where mutual shading may affect the light reaching the canopy at lower levels (Tewolde et al., 2018). Several studies have already demonstrated that the application of intracanopy LED, also named interlighting, can increase yield and other quality aspects of greenhouse tomato production, affecting plant photosynthesis and plants photoreceptors responses (Appolloni et al., 2021a). These studies are mostly concentrated in countries of cold regions, where light limitation makes this practice crucial and more cost-effective. However, an application in the Mediterranean area could also be of interest in case of an extension of production during the winter period (Palmitessa et al., 2021a), when the market could guarantee higher selling prices, while environmental conditions may limit productive capacity. Furthermore, interesting application for lower latitude countries may also be related to the need to whitewash greenhouses to protect plants from excessive heat during the hot period (Palmitessa et al., 2021a).

1.2 Light Emitting Diodes (LEDs) for tomato production

Cloud cover, low sun radiation, as well as mutual shading in case of high plant density, can affect photosynthesis and carbon sequestration with consequent limiting effects on crops productivity (Tewolde at al., 2018; Zhang et al., 2015). Supplemental lighting in greenhouse horticulture have been increasing application thanks to actual potentialities overcoming these limitations, evolving technology from lower efficient conventional lamps to increasingly capable solutions (Pinho et al., 2012; Gupta and Agarwal, 2017). Although numerous lamps typologies, including fluorescent or high-pressure sodium lamps, can be used as effective supplemental lighting sources for plant cultivation (Lu et al., 2012a; Pan et al., 2019), their efficiency compared to current Light Emitting Diodes (LEDs) is limited.

Nowadays, LED lamps represent the most advantageous mean of artificial lighting in terms of electrical-use efficiency, expecting to reduce consumption costs (Olle and Viršile, 2013). Their benefits also refers to cultivation management aspects, particularly involving possible miniaturization, low weight and limited radiant heat emittance (Ibaraki, 2017). Indeed, small sized lamps can not only minimize the interference of natural sun light, but also ameliorate mobility allowing for a light supply following plants growth phases (Ibaraki, 2016). Furthermore, they can be used in close proximity to plant canopy without excessively increasing leaf temperature (Morrow, 2008), therefore being also applicable as interlighting to reduce intracanopy shading conditions in different high-stem species (Jokinen et al., 2012, Gómez and Mitchell, 2016a; Kumar et al., 2016). Furthermore, LED lamps can have longer life span compared to other lamp typologies, achieving 50’000 hours before starting to dim their original intensity by 70% (Bourget, 2008; Olle and Viršile, 2013).

Beside management advantages, LEDs can favor the application of tailored light quality (light spectrum) and quantity (light intensity) depending on cultivated crop. In fact, being easily associable to digital control systems, different spectra and intensities can be programmed depending on photoperiod or developing stage (Yeh and Chung, 2009; Olle and Viršile, 2013). Setting of specific wavelengths can determine relevant effects not only on plant photosynthesis, but also on morphogenesis or other physiological aspects (Ouzounis et al., 2015; Ibaraki, 2017). For instance, Red wavelength can favor flowers development (Liao et al. 2014), while Blue or UV spectrum can increase plant protection from diseases (Tokuno et al., 2012; Hui et al., 2017) also preserving post-harvest quality and food safety through inactivation of pathogens action (D'Souza et al., 2015). Moreover, specific light spectra can ameliorate qualitative and nutraceutical aspects such as antioxidant components content (e.g., flavonoids, ascorbic acid) of various species (Ebisawa et al., 2008; Carvalho et al., 2016; Jiang et al., 2017, Mempel and Wittmann, 2019), being related to photoreceptors activation (Alba et al., 2000). Finally, the application of narrow LED wavelengths in greenhouse condition may represent not only a direct advantage on plants production, but also a useful tool for Integrated Pest Management (IPM) of harmful arthropods (Johansen et al., 2011). Finally, potential application may also be related to post-harvest quality (Affandi et al., 2021; Castillejo et al., 2021).

Supplemental LED lighting can find an interesting commercial application in the case of greenhouse tomato (*Solanum lycopersicum*) production, being one of the most relevant horticultural crops worldwide (Deram et al., 2014; FAOSTAT, 2018). Intensive greenhouse production normally applies high-wire productive systems, which can determine labor reduction, multi-harvests and possible automation (Giniger et al., 1988; Okano et al., 2001). Nonetheless, high plant density required for this system can limit light penetration within canopy with consequences on fruit quality and yield (Wada et al., 2006). In this context, LEDs have already been demonstrated to be a useful source of additional light both for qualitative and quantitative improvement of greenhouse truss-tomato production by numerous researches (Tewolde et al, 2016; Dzakovich et al. 2017; Jiang et al., 2017; Kim et al., 2019) (**Figure 2**). However, some inconsistencies of results among studies have been showed, reporting non-significant effects of artificial light, especially in case of qualitative parameters (e.g., soluble solid, ascorbic acid) (Lu et al., 2012b; Hao et al., 2016).

Figure 2. Tomato cultivation with supplemental LED light with an intensive soilless high-wire system (Pellerossa I-Pom, Budrio, Bologna, Italy) (Photo by Ivan Paucek).

1.3 Building-integrated Agriculture (BIA) and LED light

Urban Agriculture (UA) is gaining increasing global interest from both governmental and non-governmental entities, as it is able to contribute actively to the achievement of several Sustainable Development Goals (SDGs) identified by the United Nations (Stevens and Kanie, 2016), and to the creation of a green economy to counter the economic and environmental crisis that the world is now facing (Bina, 2013). Indeed, as more than half of the global population currently lives in cities (FAO-FCIT, 2018), urban areas play a central role in achieving a sustainable growth. Furthermore, the recent historical events, such as COVID-19 pandemic and geo-political tensions, have shown the need to create more resilient food systems capable of ensuring food self-sufficiency (Rivera-Ferre et al., 2021). As competition for urban land use and the resulting excessive costs may limit on-soil UA, the exploitation of unused city spaces or vertical structure may be a solution to overcoming this barrier to development.

Building-Integrated Agriculture (BIA) is a form of UA applied in or on top of buildings (Specht et al., 2015) (**Figure 3**). In the first case, it applies indoor cultivation systems, characterized by the use of artificial LED light. When performed on more layers and in controlled environment conditions, it assumes the name of Vertical Farm or Plant Factory with Artificial Lighting (PFALs) (Kozai, 2019). In case of cultivation on top of buildings, BIA is called Rooftop Agriculture (RA), and can be applied in protected (rooftop greenhouses) or non-protected (open-air) conditions (Orsini et al., 2017). When a rooftop greenhouse is connected to the building, mutually exchanging heat, water and CO_2, it is called Integrated Rooftop Greenhouse (i-RTG), due to the integration of the building and greenhouse metabolisms (Nadal et al., 2017). This integration can lead to a strong sustainability impact, favoring the optimization and recycle of cultivation inputs, energy saving, and emission reduction (Montero et al., 2017; Nadal et al., 2017). Unlike indoor cultivation, RA may not need the use of artificial light, given the exposure to natural sunlight. However, the shading of surrounding buildings, as well as bulky structural items and loss of transmissivity of fireproof covering materials (e.g., polycarbonate) imposed by municipality structural and fire safety codes (Muñoz-Liesa et al., 2021), may the determine the need of supplemental LED light application to guarantee adequate photosynthesis and production.

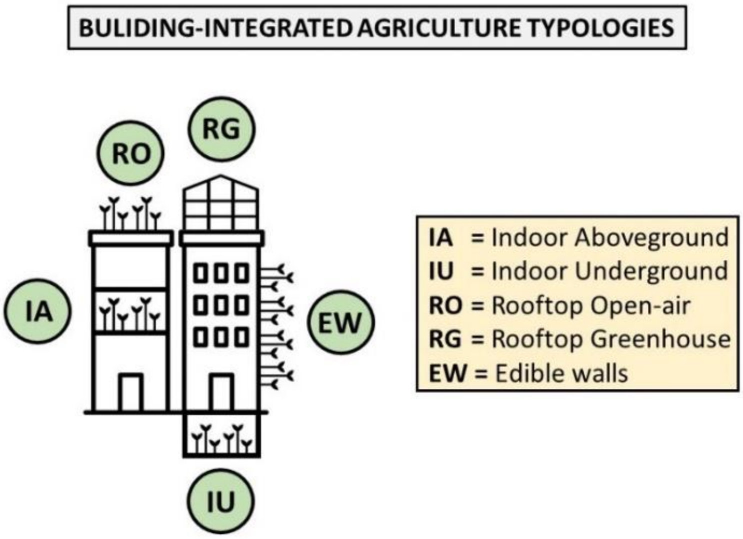


Figure 3. Typologies of Building-Integrated Agriculture (BIA).

1.4 Aim of the thesis

Greenhouse tomato cultivation using supplemental LED light has already gained wide interest by research, especially in high latitude countries where this technique can help overcoming light limitations. However, the field of application of supplemental LED light are still numerous and need to be explored to guarantee an innovation upgrade and amelioration of production, also in lower latitude countries. The aim of the thesis was to investigate diversified applications of supplemental LED interlighting for greenhouse tomato production (*Solanum lycopersicum*), with a specific focus on the Mediterranean context. To target this aim, the research was developed basing on the following research questions:

- **RQ1**: What is the current state of tomato cultivation with additional LED light and what aspects still need to be explored? (**Chapter 1** and **2**).
- **RQ2**: What is the potential of supplemental LED light to reduce structural shadings and limited transmission of solar radiation in case of tomato cultivation in i-RTGs? (**Chapter 3**).
- **RQ3**: Could supplemental LED light during cultivation affect post-harvest quality of tomato? (**Chapter 4**).
- **RQ4**: Beside fruit production, could the application of supplemental LED light interest other commercial areas such as tomato seedlings production? (**Chapter 5**).
- **RQ5**: Could the use of additional LED light influence some management aspects of tomato cultivation such as defoliation, whit consequences on qualitative and quantitative aspects of production? (**Chapter 6**).

2 MATERILAS AND METHODS

The research was developed in two phases:

1. a first phase of definition and in-depth analysis of the research framework. During this phase a meta-analysis was carried out on the use of supplementary LED light for the greenhouse cultivation of tomato (**Chapter 1**), and a systematic review on the worldwide development RA, as a potential sector to improve also using additional LED light (**Chapter 2**);
2. a second phase of experimental application. During this phase, four trials were carried out (**Chapter 3**, **4**, **5** and **6**) building upon the observations of previous phase, which highlighted underexplored fields of application.

The **Figure 4** resumes the phases of thesis development. Further details on Materials and Methods applied are reported in the following paragraphs.

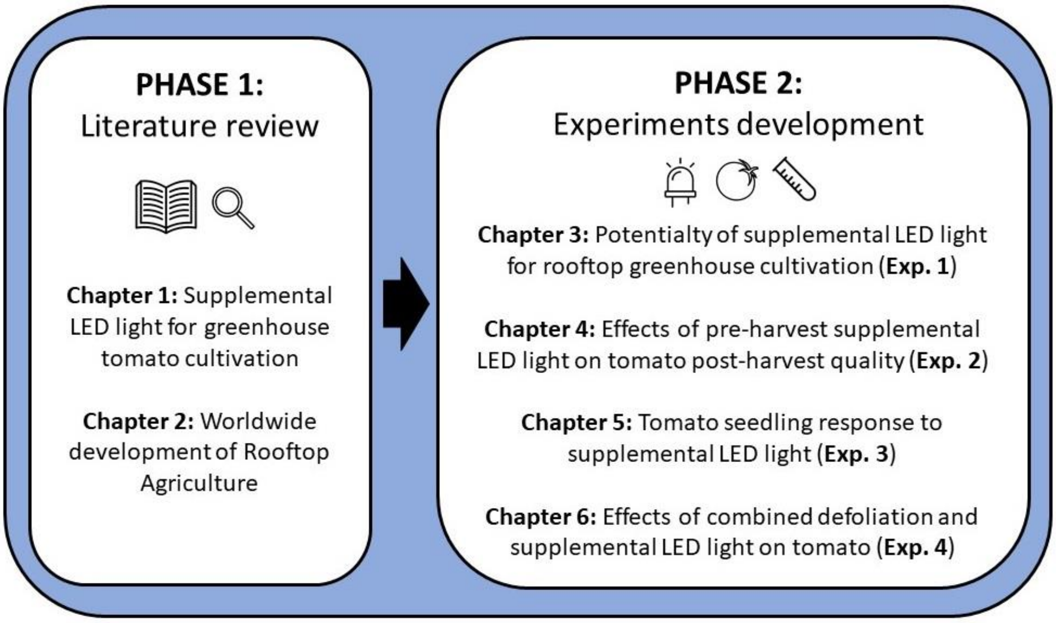


Figure 4. Resume of thesis development phases.

2.1 Phase 1: Definition of research framework

During the first phase, which mainly took place during the first year of PhD at the University of Bologna, two literature reviews were carried out, with the aim of (**1**) defining the state of the art of the research topic and the working framework, and (**2**) identifying possible topics and sectors not yet explored or with a margin for improvement. The two reviews responded to **RQ1**.

In particular, the two literature reviews were:

1. a systematic review of the literature available at the time of the research on the use of supplementary LED light for greenhouse cultivation of tomato, deepened with a meta-analysis of the main outputs of published papers (**Chapter 1**);
2. a systematic review of the cases of RA in the world, to present a general picture of the sector, define the different typologies and identify topics for future development (**Chapter 2**).

2.1.1 Review 1: Evaluating the current status of supplemental LED light for greenhouse tomato cultivation

The first review on the subject of the use of supplementary LED light for greenhouse tomato cultivation was used to identify all the research available at the time of the evaluation, to observe the state of the art of the sector and topics not yet or limitedly explored. Furthermore, a statistical analysis of the main research outputs made it possible to evaluate the actual potential of this cultivation technique. Two analytical methods were respectively applied to carry out this evaluation: a systematic review and a meta-data analysis.

Systematic review:

The systematic review was carried out during the first half of 2020 using online databases (e.g., Google Scholar and Scopus), and applying the following search string: LED AND supplemental light* AND tomato* AND greenhouse. Only accessible published material in English language was collected, including scientific articles, conference papers, book chapters and thesis dissertations. The collection of articles only considered *Solanum lycopersicum*, in which additional LED light was applied for long term period. During this phase, general data were collected form the papers, including: experiment design and environmental features (cultivar, location, maximum and minimum temperatures, relative humidity, nutrient solution electrical conductivity (EC) and pH, plant density, greenhouse typology and growing system), and LED treatment characteristics (light spectrum, intensity and photoperiod, treatment duration and other eventual specific experimental conditions, e.g., nighttime treatments). This data were evaluated using descriptive statistic.

Meta-data analysis:

Meta-data analysis is a systematic evaluation method based on statistical analysis, applied on the same variables coming from different researches, but evaluated and collected with similar methods. The meta-data analysis is based on outcomes evaluation, also called the effect sizes or response ratios [R] (Hedges et al., 1999). The outcomes used for the analysis were: fresh fruit mass yield (yield, expressed as kg plant^{-1} of fresh fruit mass), soluble solid content (TSS, expressed as °Brix), ascorbic acid content (Asc, expressed as mg Asc 100 g^{-1} of fruit fresh weight), chlorophyll content (Chl, expressed as Chl index), net photosynthesis (PN, expressed as µmol CO_2 m^{-2} s^{-1}), stomatal conductance (gs, expressed as mmol H_2O m^{-2} s^{-1}) and leaf area (LA, expressed as m^2 per plant). . Each study can account for more than one treatment; therefore, each treatment was evaluated as a separate observation (k). The [R] of each observation was calculated as follows:

$$\ln \mathrm{R} = \ln (\mathrm{R}) = \frac{\ln \mathrm{m_t}}{\ln \mathrm{m_c}} = \ln \mathrm{m_t} - \ln \mathrm{m_c}$$

where m_t and m_c represent the mean outcomes of the treatment and control, respectively (Hedges et al., 1999; Borenstein et al., 2009). Since most of the considered publications did not display standard error (SE), variance (Var) or standard deviation (S) values, an unweighted meta-analysis was applied to equally weight each observation (McDaniel et al., 2014; Qin et al., 2015). The data were analyzed using the online available software Meta-Essential (Suurmond et al., 2017). A subgroup analysis was also performed. The [R] value was accepted as significant with a p-value ≥0.05. Further details on review analysis are reported in **Chapter 1**.

2.1.2 Review 2: An overview of RA sector

The second review was performed to evaluate the worldwide development of RA and analyze the diversification of the sector. This review served to identify potential areas of development, for supplemental LED light application as well. The evaluation was performed applying a systematic review of worldwide cases of RA.

Systematic review:

The systematic review was built upon an already existing database compiled in 2011-2012 (Thomaier et al., 2015) that was updated and enlarged between 2017 and 2019. The update was performed by using two inventories: the Rooftop Agriculture Handbook by Orsini et al.(2017) and the scientific article by Sanyé-Mengual et al. (2017). The update continue by investigated scientific and gray literature as well as websites, to identify those RA cases not inventoried in the previous sources. In this case, the search was performed using the following search terms: “rooftop agriculture”, “rooftop farming”, “rooftop garden”, “rooftop greenhouse”, “building-based agriculture”, “zero-acreage farming”, “rooftop aquaponics” and “building agriculture”. The type of data collected included both general and operational aspects. General aspects were: Rooftop Agriculture type (Open-air or Rooftop Greenhouse), building type and farming purpose. Operational aspects were: basic and structural observations (starting and closing date, size, activities performed, typology of organization), agronomical parameters (growing system, type of crop/product, crop yield), resource use (water source, energy source, nutrient typology, nutrient form), social and economic aspects (members, women members, population at risk of social exclusion, costs, income, installation costs), societal impact (consumers, visits, trainees, users), and sustainability actions (e.g., use of renewable energy, use of chemicals, recycle of nutrients). This data were evaluated using descriptive statistic. Further details on review analysis are reported in **Chapter 2**.

2.2 Phase 2: Experiments development

This phase was mainly carried out during the second and third years of the PhD, taking place in two research centers: the Department of Agro-Food Sciences and Technologies of the University of Bologna (DISTAL - UNIBO), Italy, and the Institut de Ciència i Tecnologia Ambientals of the Universitat Autònoma de Barcelona (ICTA - UAB), Spain. During this phase, four experiments were developed with the aim of deepening underexplored topics of interest on the application of supplementary LED light for the cultivation of tomatoes in greenhouses, emerged during the first phase of the research. The four experiments responded to **RQ2**, **3**, **4** and **5**.

The experiments (Exp.) performed consisted in:Evaluation of the potential of supplemental LED light to improve tomato production under the sub-optimal lighting condition of an i-RTG (**Exp. 1** - **Chapter 3** - **RQ2**) (**Figure 5**);

Figure 5. Cultivation of tomato using supplemental LED light in the i-RTG at ICTA-UAB.

Evaluation of the effect of supplemental LED light applied during cultivation on post-harvest quality of tomato (**Exp. 2** - **Chapter 4** - **RQ3**) (**Figure 6**);

Figure 6. Cultivation of tomatoes with supplemental LED light in the commercial greenhouse Pellerossa I-Pom, Budrio, Bologna, Italy.

1. Evaluation of tomato seedlings growth response under different qualities of supplemental LED light (**Exp. 3** - **Chapter 5** - **RQ4**) (**Figure 7**);

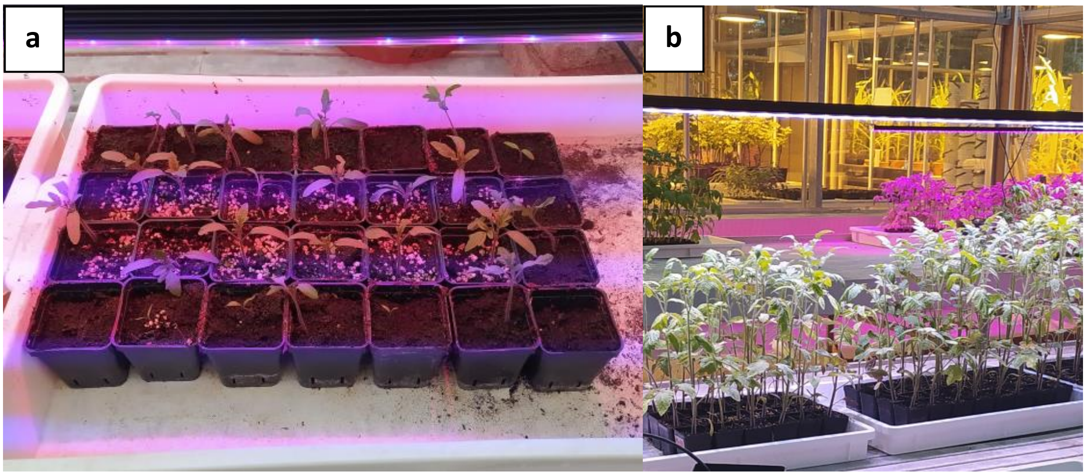


Figure 7. Tomato seedlings cultivation under supplemental LED light in the experimental greenhouse at DISTAL - UNIBO. **(a)** Treatment application during first stages of development; **(b)** treatment application during last stages of development.

2. Evaluation of the effect of defoliation combined with supplemental LED lighting on tomato production (**Exp. 4** - **Chapter 6** - **RQ5**) (**Figure 8**).

Figure 8. Combination of defoliation regimes and light treatments on tomato in the in the experimental greenhouse at DISTAL - UNIBO. **(a)** Treatment application during nighttime hours; **(b)** treatment application during daytime hours.

2.2.1 Growing system and experiments location

The **Exp. 1**, **2** and **4** were performed using a high-wire soilless system, with slight modifications as reported below. The **Exp. 3** was performed growing tomato seedlings in pots filled with peat and irrigated manually. The variety of tomato used in each experiment was *Solanum lycopersicum* cv. *Siranzo* (Rijk Zwaan Zaadteelt en Zaadhandel B.V, De Lier, Netherlands). The climatic control was done automatically, using specific sensors available in the different infrastructures, monitoring temperature, relative humidity, and solar radiation values.

The infrastructures and periods of each experiment are reported as follow (**Table 1**):

- **Exp. 1** (**Chapter 3**): it was performed in the i-RTG of the Institut de Ciència i Tecnologia Ambientals of the Universitat Autònoma de Barcelona (ICTA - UAB), Spain, during April-July 2021. The plants were cultivated in perlite bags with a drip irrigation system recirculating the nutritive solution.
- **Exp. 2** (**Chapter 4**): the first part was performed in the commercial greenhouse "Pellerossa, I-Pom" in Budrio (Bologna), Italy, using an intensive high-wire cultivation system with rockwool slabs and a drip irrigation system recirculating the nutritive solution. The second part was continued in the cold chamber of the experimental farm of the University of Bologna in Cadriano (Bologna), Italy, were red-ripen tomatoes were kept in the dark for 1 week at 13°C and 80% of RH. The entire experiment lasted from the end of August to November 2020.
- **Exp. 3** (**Chapter 5**): it was performed in the experimental greenhouse of the Department of Agro-Food Sciences and Technologies of the University of Bologna (DISTAL - UNIBO), Italy, during March-June 2021.
- **Exp. 4** (**Chapter 6**): it was performed in the experimental greenhouse of the Department of Agro-Food Sciences and Technologies of the University of Bologna (DISTAL - UNIBO), Italy, during September-February 2021-2022. The plants were cultivated in perlite bags using an open-drip irrigation system.

Table 1. Resume of experiments location and period.

EXPERIMENTS	LOCATION	PERIOD
Exp. 1 (**Chapter 3**)	Barcelona, Spain	April-July 2021
Exp. 2 (**Chapter 4**)	Bologna, Italy	August-November 2020
Exp. 3 (**Chapter 5**)	Bologna, Italy	March-June 2021
Exp. 4 (**Chapter 6**)	Bologna, Italy	September-February 2021-2022

2.2.2 Lighting treatments

Except for **Exp. 4**, all the other **Exp.** (**1**, **2** and **3**) applied the following light spectra (**Figure 9**):

1. Red and Blue in a 3 ratio (RB);
2. Red and Blue in a 3 ratio + Far-Red the whole day (FR);
3. Red and Blue in a 3 ratio + Far-Red at the end-of-day for 30 min after the end of RB treatment (EOD).

A control grown under natural light only was also considered (CK). **Exp. 4** only applied the RB and CK treatment, combining it with two defoliation regimes: removal before harvesting at turning stage (R), and non-removal for the entire cultivation period (NR). All light treatments were added to natural sunlight for 16 h d^{-1} (8-00 am), with an intensity and position that slightly varied according to the experiment:

- **Exp. 1**, 170 µmol m^{-2} s^{-1} applied with a double LED interlighting lamp (Philips GreenPower LED, Philips, Amsterdam, The Netherlands);
- **Exp. 2**, 180 µmol m^{-2} s^{-1} applied with single LED interlighting lamp (Flygrow Interlight, Flytech LED Technology, Belluno, Italy);
- **Exp. 3**, 180 µmol m^{-2} s^{-1} applied with single LED interlighting lamp (Flygrow Interlight, Flytech LED Technology, Belluno, Italy), but used as top light;
- **Exp. 4**, 180 µmol m^{-2} s^{-1} applied with single LED interlighting lamp (Flygrow Interlight, Flytech LED Technology, Belluno, Italy).

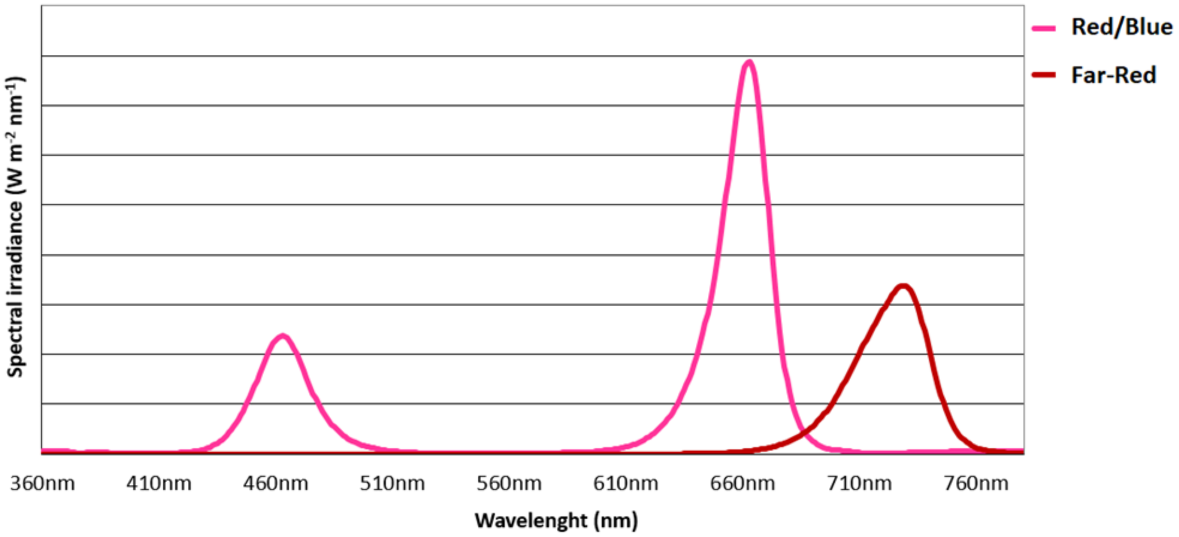


Figure 9. Peak wavelength (nm) of emitted spectrum according to spectral irradiance (W m^{-2} nm^{-1}).

2.2.3 Measured parameters

During the four experiments different vegetative, physiological, biochemical and qualitative evaluations were performed. **Table 2** reports a resume of the evaluated variables for each experiment.

Table 2. Resume of evaluated parameters for each experiment.

	Exp. 1	**Exp. 2**	**Exp. 3**	**Exp. 4**
Vegetative evaluations				
Apical growth	X			
Internode length	X			
Stem length				X
Stem diameter	X			
Hypocotyl length			X	
Collar diameter	X			X
Leaf area	X		X	X
Leaf fresh weight	X		X	X
Leaf dry weight	X		X	X
Stem fresh weight	X		X	X
Stem dry weight			X	X
Fruit fresh weight	X	X		X
Fruit dry weight	X			X
Fruit diameters (equatorial and polar)	X			X
Fruit ripening	X	X		X
Fruit setting				X
Growth indexes				
Relative Growth Rate (RGR)			X	
Leaf Area Ration (LAR)			X	
Specific Leaf Area (SLA)	X		X	X
Leaf Weight Ratio (LWR)			X	
Net Assimilation Rate (NAR)			X	
Physiological evaluations				
Chlorophyll content	X		X	X
PSII quantum efficiency				X
Under-leaf CO_2 concentration				X
Stomatal conductance				X
Leaf transpiration				X
Net photosynthesis				X
Biochemical evaluations				

Lycopene	X	X		X
β-carotene	X	X		X
Total phenols content	X	X		
Antioxidant capacity	X	X		
Micro-macronutrients analysis	X			
Qualitative evaluations				
Fruit hardness	X	X		X
Pulp firmness	X			X
Color determinations	X			X
Soluble solids	X	X		X
Acidity	X	X		X
Chilling injury evaluation	X			
Economic evaluation	X			X

2.2.3.1 Vegetative evaluations

Internode length was measured as the distance between two consecutive fruit trusses. Stem and collar diameter were measured with a digital caliper 1 cm under the fruit truss and 1 cm from the point of attachment to the cultivation substrate, respectively. Fruit polar and equatorial diameters were measured with a digital caliper. Fresh and dry weights of leaves, stems and fruits were measured with a digital scale. Fruit setting was evaluated by counting the initial number of productive units (flowers and buds) and confronting it with the final number of fruits. Leaf area was measured using Easy Leaf Area software (Department of Plant Sciences, University of California, Davis, CA, USA) in case of **Exp. 1**; a leaf area meter (LI-3100C Area Meter, LI-COR Biosciences, Lincoln, United States) in case **of Exp. 4**; and a photographic scanner (Epson Perfection v370, Seiko Epson Corporation, Suwa, Nagano Prefecture, Japan), Adobe Photoshop 2019 and ImageJ v. 1.8.0. in case of **Exp. 3**. Fruit ripening was measured using a DA-Meter (SINTELEIA, Bologna, Italy). More details on vegetative evaluations are reported in Materials and Methods of **Chapter 3**, **4**, **5** and **6**.

2.2.3.2 Growth indexes

The Relative Growth Rate (RGR) represents the rate of growth of the plant relative to its total weight, expressed in $g\ g^{-1}d^{-1}$ (Williams, 1946). This index is calculated as:

$$\mathrm{RGR} = \frac{\overline{\ln \mathrm{W}_2} - \overline{\ln \mathrm{W}_1}}{\mathrm{t}_2 - \mathrm{t}_1}$$

where W_1 and W_2 are the total dry weights of the plants at times t_1 and t_2. The Net Assimilation Rate (NAR) indicates the growth rate of the crop in relation to the leaf growth unit, expressed in $g\ cm^{-2}\cdot d^{-1}$. It is an index of photosynthetic efficiency, closely dependent on the genetic characteristics of the crop as well as its ability to intercept light radiation (Williams, 1946). This index was calculated as follows:

$$\mathrm{NAR} = \frac{\ln \mathrm{A}_2 - \ln \mathrm{A}_1}{\mathrm{A}_2 - \mathrm{A}_1} \cdot \frac{\mathrm{W}_2 - \mathrm{W}_1}{\mathrm{t}_2 - \mathrm{t}_1}$$

where A_1 and A_2 are the leaf area at t_1 and t_2. The Leaf Area Ratio (LAR) indicates the ratio between the area of the leaf and the total plant biomass, expressed in cm^2 g^{-1}. This index represents the amount of leaf area formed as a result of each unit of biomass accumulated by the plant and is, therefore, an index of the light interception capacity of the crop (Radford, 1967). This index was calculated as follows

$$\mathrm{LAR} = \frac{\mathrm{A_2} - \mathrm{A_1}}{\ln \mathrm{A_2} - \ln \mathrm{A_1}} \cdot \frac{\ln \mathrm{W_2} - \ln \mathrm{W_1}}{\mathrm{W_2} - \mathrm{W_1}}$$

The Leaf Weight Ration (LWR) indicates the ratio of the dry weight of leaves to that of the whole plant, expressed in g g^{-1} (Kvet, et al., 1971). This index was calculated as follows:

$$\mathrm{LWR} = \frac{\mathrm{L_2} - \mathrm{L_1}}{\ln \mathrm{L_2} - \ln \mathrm{L_1}} \cdot \frac{\ln \mathrm{W_2} - \ln \mathrm{W_1}}{\mathrm{W_2} - \mathrm{W_1}}$$

Where L_1 and L_2 are the leaf dry weights at t_1 and t_2. Finally, the Specific Leaf Area (SLA) indicates the ratio of leaf area to leaf dry weight, expressed in cm^2 g-^{1} (Kvet, et al., 1971), a low value of SLA is therefore indicative of a high leaf thickness. This index was calculated as follows:

$$\mathrm{SLA} = \frac{\mathrm{A_2} - \mathrm{A_1}}{\ln \mathrm{A_2} - \ln \mathrm{A_1}} \cdot \frac{\ln \mathrm{L_2} - \ln \mathrm{L_1}}{\mathrm{L_2} - \mathrm{L_1}}$$

2.2.3.3 Physiological evaluations

Chlorophyll content of leaves was evaluated with a SPAD-502PLUS (Konica Minolta, Tokyo, Japan) in case of **Exp.3** and **4**, while during **Exp. 1** a Chlorophyll Content Meter CCM-200 (Opti-Sciences, Hudson, NY, USA) was used. Stomatal conductance (GS, in mmol m^{-2} s^{-1}), under-leaf CO_2 concentration (Ci), leaf transpiration (E, in mmol m^{-2} s^{-1}), net photosynthesis (A, in µmol m^{-2} s^{-1}), and effective quantum yield efficiency of PSII (ΦPSII), were measured using a portable LI-COR 6400 (LI-COR Biosciences, Lincoln, United States), set as reported by Calone et al., 2021.

2.2.3.4 Biochemical evaluations

Lycopene and β-carotene content were evaluated on frozen tomato samples, using the methodology described by Anthon and Barrett (2006). The following formulas were used to calculate the content:

$$\text{lycopene} \left(\frac{\mathrm{mg}}{\mathrm{kg}}\right) = \left(\frac{\mathrm{X}}{\mathrm{Y}}\right) \times \mathrm{A503} \times 3.12$$

where X is the amount of hexane (mL), Y the weight of the fruit tissue (g), A503 is the absorbance at 503 nm, and 3.12 is the extinction coefficient.

$$\beta\text{- carotene} = (9.38 \times \mathrm{A444} - 6.70 \times \mathrm{A503}) \times 0.55 \times 537 \times \frac{\mathrm{V}}{\mathrm{W}}$$

where A444 is the absorbance at 444 nm, A503 is the absorbance at 503 nm, 0.55 is the ratio of the final hexane layer volume to the volume of mixed solvents added for hexane:acetone:ethanol

(2:1:1), V is the volume of mixed solvents added, W is the fresh weight of the sample, and 537 (g $mole^{-1}$) is the molecular weight of β-carotene.

Total antioxidant capacity was analyzed on frozen tomatoes samples by using the FRAP (Ferric Reducing Antioxidant Power) method, developed following the method described by Benzie and Strain (1999). FRAP values were expressed as mmol Fe^{2+} kg^{-1} FW.

Total polyphenols were determined on frozen tomatoes samples using the methodology described by Waterhouse (2002). The total phenolic content was expressed as gallic acid equivalent in milligram per 100 g of fresh weight (mg GA 100 g^{-1} FW).

The analysis of lycopene, β-carotene, antioxidant capacity and total phenols content of **Exp.1** were performed in the infrastructures of UAB, Barcelona, Spain; while the analysis of **Exp. 2** and **4** were performed in the laboratories of UNIBO, Bologna, Italy. In the first case, the extracted samples were read with a spectrophotometer 8453 UV-Visible, HP, Palo Alto, United States; while in the second case were evaluated with a spectrophotometer Biochrom Ltd, Cambridge, England.

Analysis of micro- and macro-elements (B, Mn, Fe, Cu, Zn, Na, Mg, K, P, S, and Ca) of plant biomass was carried out through a digestion process with HNO_3 and analyzed using ICP-OES optical spectrometry (Optima 4300DV, Perkin-Elmer, Waltham, MA, USA) as reported in Arcas-Pilz et al. (2021). The analysis were performed in the laboratory of ICTA-UAB, Barcelona, Spain.

2.2.3.5 Qualitative evaluations

Fruit hardness was evaluated by using a Durofel device (Giraud Technologies, Cavaillon, France) in case of **Exp. 2** and **4**, and by using a fruit hardness tester (Turoni®, Forlì, Italy) in case of **Exp. 1**. Fruit firmness was evaluated with a fruit texture analyzer (FTA GÜSS, Strand, South Africa). Soluble solids content and titratable acidy of **Exp. 2** and **4** were evaluates with a digital refractometer model PAL-1 (Atago Co., Ltd., Tokyo, Japan) and an automatic TitroMatic (Compact-S titrator, Crison, Modena, Italy), respectively; while a pocket Brix and acidity meter (PAL-BX|ACID3, Atago, Tokyo, Japan) was used to evaluate both parameters in case of **Exp. 1**. A colorimeter (Chroma Meter CR-400, Minolta, Tokyo, Japan) was used to assess color determinations.

2.2.3.6 Chilling injury evaluation

Chilling injury was evaluated on red and mature-green tomatoes stored at 4°C in the dark for one week, and subsequently moved in a dark room at 20°C for two weeks with 60% RH and 600–660 ppm of CO_2. Chilling injury index was visually attributed after two weeks at 20°C according to Vega-Garcìa et al. (2010) and Affandi et al. (2020).

2.2.3.7 Economic evaluations

The economic evaluation was based on energy consumed by a lamp and confronted with the electricity prices. The price of electricity was acquired from EUROSTAT (2021).

2.2.4 Statistical analysis

The statistical analysis of **Exp. 1**, **2** and **3** were performed with a one-way ANOVA; while in **Exp. 4** were performed with a two-way ANOVA. Post-hoc Tukey's Test was applied. Differences were considered significant with a p-value ≥ 0.05. Data were analyzed by using SPSS software.

3 RESULTS

This section will report the researches developed during the thesis divided by Chapters. The division by Chapters is reported below:

- **Chapter 1:** Supplemental LED lighting effectively enhances the yield and quality of greenhouse truss tomato production: results of a meta-analysis.
  Published article: Appolloni, E., Orsini, F., Pennisi, G., Gabarrell Durany, X., Paucek, I., Gianquinto, G. (2021). Supplemental LED lighting effectively enhances the yield and quality of greenhouse truss tomato production: results of a meta-analysis. Frontiers in plant science, 12, 596927. doi: 10.3389/fpls.2021.596927;

- **Chapter 2:** The global rise of urban rooftop agriculture: a review of worldwide cases.
  Published article: Appolloni, E., Orsini, F., Specht, K., Thomaier, S., Sanye-Mengual, E., Pennisi, G., Gianquinto, G. (2021). The global rise of urban rooftop agriculture: A review of worldwide cases. Journal of Cleaner Production, 296, 126556. doi: 10.1016/j.jclepro.2021.126556;

- **Chapter 3:** Supplemental LED Lighting Improves Fruit Growth and Yield of Tomato Grown under the Sub-Optimal Lighting Condition of a Building Integrated Rooftop Greenhouse (i-RTG).
  Published article: Appolloni, E., Paucek, I., Pennisi, G., Stringari, G., Gabarrell Durany, X., Orsini, F., Gianquinto, G. (2022). Supplemental LED lighting improves fruit growth and yield of tomato grown under the sub-optimal lighting condition of a building Integrated Rooftop Greenhouse (i-RTG). Horticulturae, 8(9), 771. doi: 10.3390/horticulturae8090771;

- **Chapter 4:** Potential application of pre-harvest LED interlighting to improve tomato quality and storability.
  Published article: Appolloni, E., Pennisi, G., Paucek, I., Cellini, A., Crepaldi, A., Spinelli, F., Orsini, F. (2023). Potential application of pre-harvest LED interlighting to improve tomato quality and storability. Postharvest Biology and Technology, 195, 112113. doi: 10.1016/j.postharvbio.2022.112113

- **Chapter 5:** Evaluation of tomato seedlings growth response under different qualities of supplemental LED light.
  Article accepted for publication;

- **Chapter 6:** Winter greenhouse tomato cultivation: matching supplementary lighting and leaf pruning for improved yield and precocity.
  Article under revison.

3.1 Chapter 1: Supplemental LED lighting effectively enhances the yield and quality of greenhouse truss tomato production: results of a meta-analysis

Elisa Appolloni[1], Francesco Orsini[1], Giuseppina Pennisi[1], Xavier Gabarrell Durany[2], Ivan Paucek[1], Giorgio Gianquinto[1]

[1]Alma Mater Studiorum – Università di Bologna, DISTAL – Department of Agricultural and Food Sciences, Bologna, Italy

[2]Universitat Autònoma de Barcelona, Institut de Ciència i Tecnologia Ambientals (ICTA-UAB), MdM Unit of Excellence; Chemical, Biological and Environmental Engineering Department, Barcelona, Spain

3.1.1 Abstract

Intensive growing systems used for greenhouse tomato production, together with light interception by cladding materials or other devices, may induce intracanopy mutual shading and create suboptimal environmental conditions for plant growth. There are a large number of published peer-reviewed studies assessing the effects of supplemental light-emitting diode (LED) lighting on improving light distribution in plant canopies, increasing crop yields and producing qualitative traits. However, the research results are often contradictory, as the lighting parameters (e.g., photoperiod, intensity and quality) and environmental conditions vary among conducted experiments. This research presents a global overview of supplemental LED lighting applications for greenhouse tomato production deepened by a meta-analysis aimed at answering the following research question: does supplemental LED lighting enhance the yield and qualitative traits of greenhouse truss tomato production? The meta-analysis was based on the differences among independent groups by comparing a control value (featuring either background solar light or solar + HPS light) with a treatment value (solar + supplemental LED light or solar + HPS + supplemental LED light, respectively) and included 34 published papers and 104 total observations. The meta-analysis results revealed the statistically significant positive effects (p-value <0.001) of supplemental LED lighting on enhancing the yield (+40%), soluble solid (+6%) and ascorbic acid (+11%) contents, leaf chlorophyll content (+31%), photosynthetic capacity (+50%) and leaf area (+9%) compared to the control conditions. In contrast, supplemental LED lighting did not show a statistically significant effect on the leaf stomatal conductance (p-value 0.171). In conclusion, in addition to some partial inconsistencies among the considered studies, the present research enables us to assert that supplemental LED lighting ameliorates the quantitative and qualitative aspects of greenhouse tomato production.

Keywords: Supplemental lighting, LEDs, Light-Emitting Diodes, Greenhouse, *Solanum lycopersicum*, Interlighting.

3.1.2 Introduction

In greenhouse tomato (*Solanum lycopersicum*) production, photosynthesis and carbon sequestration may be hindered by cloud cover, shading systems and variable solar radiation, as well as by plant mutual shading (e.g., when high vertical stem training or increased crop densities are used) (Zhang et al., 2015; Tewolde et al., 2018). Considering the nonuniform distribution of solar radiation around the world, limitations may also occur in cases of high-latitude countries such as Canada, Japan, Norway, as well as in the northern areas of China and the United States, where long winters and low DLIs (daily light integrals) may affect greenhouse production (Garland et al., 2010; Deram et al., 2014; Sun et al., 2015; Tewolde et al., 2016; Paponov et al., 2020). Supplemental artificial lighting can be applied to increase greenhouse yields and ensure stable year-round production regardless of environmental conditions (Ohashi-Kaneko et al., 2007), even in regions with high DLIs, such as the Mediterranean and Jordan Valley (Israel) (Joshi et al., 2019; Paucek et al., 2020). Today, light-emitting diode (LED) lamps represent the most advantageous artificial lighting systems in terms of energy use efficiency, with foreseen expectations for further reducing investments and running costs in the near future (Olle and Viršile, 2013). Additional advantages also involve the functional aspects of LEDs that make the technology suitable for cultivation, particularly thanks to their possible miniaturization, light weight and limited radiant heat emissions (Ibaraki, 2017). Accordingly, LED lamps can be used in proximity to plant canopies without excessively increasing the leaf temperature (Morrow, 2008), enabling inter-lighting applications that reduce intracanopy shading conditions in high-stem-density plants (Jokinen et al., 2012, Gómez and Mitchell, 2016a; Kumar et al., 2016; Hao et al., 2017).

LED application can enable the fine tuning of combinations between light spectral compositions and light intensities, with direct consequences not only on yield but also on structural and physiological plant aspects (Ouzounis et al., 2015; Hao et al., 2017; Ibaraki, 2017). In fact, the responses of plants to light characteristics are regulated by photoreceptors that reading specific wavelengths, intensities or photoperiods can trigger signals that modify plant metabolism (Christie, 2015). Accordingly, light environmental management can lead to interesting commercial results. For instance, Red light can promote flower development (Liao et al. 2014), while the Blue-violet spectrum can increase plant protection from diseases (Tokuno et al., 2012; Hui et al., 2017), preserving postharvest conservation and food safety through the inactivation of pathogen action (D'Souza et al., 2015). Moreover, specific light spectra can improve the qualitative and nutraceutical aspects of plants (Mempel and Wittmann, 2019), such as enhancing antioxidant compound biosynthesis (e.g., flavonoids, ascorbic acid) in various species (e.g., lettuce, basil, tomato) (Ebisawa et al., 2008; Carvalho et al., 2016; Jiang et al., 2017; Pennisi et al., 2019a, Pennisi et al., 2019b).

Stomatal conductance is a specific physiological response that is guided by light. The wavelength mainly involved in this process is Blue light (450 and 495 nm), which is also implicated in other mechanisms, such as phototropism, chloroplast migration, photomorphogenesis and chlorophyll production (O'Carrigan et al., 2014b). Cryptochromes and phototropins are the main photoreceptors stimulated by Blue light (Christie, 2015); these photoreceptors go through a phosphorylation process and bind protein to trigger proton extrusion and K^+ uptake in stomatal guard cells, with the consequent cell turgidity and stomatal opening enabling gas exchange (Roelfsema and Hedrich, 2005: Shimazaki et al., 2007). Apparently, Green and Red light may also play roles in gas exchange by inducing stomatal closure, as Green light may stop soluble uptake in guard cells (Talbott et al., 2002), while Red light may lead to K^+ and solute losses (Zeiger, 1990). In tomato plants, studies that have applied Blue, Red and Green lighting in closed chambers seem to confirm such observations (O'Carrigan et al., 2014b; Bian et al., 2019), opening the possibility of integrating Green LED lighting to reduce drought stress in tomato plants (Bian et al., 2019). However, it is important to consider that what is observed in growing chamber experiments is not always transferable to the processes occurring in productive systems, where different environmental factors may affect plant responses.

Greenhouse tomatoes represent one of the most relevant horticultural crops worldwide (Deram et al., 2014; FAOSTAT 2018). In intensive greenhouse tomato production, high-wire single-truss training systems are normally applied to enable labor reductions, multiple harvests and possible automation (Giniger et al., 1988; Okano et al., 2001). Nonetheless, the high plant density required for these systems can limit light penetration within canopies with consequences on fruit quality and yield (Wada et al., 2006). In this context, several studies have already reported the usefulness of LED lighting system applications for qualitative and quantitative improvements in greenhouse truss tomato production (Tewolde et al, 2016; Dzakovich et al. 2017; Jiang et al., 2017; Kim et al., 2019). However, inconsistencies among studies are also present; nonsignificant effects of supplemental LED lighting, especially on qualitative parameters (e.g., soluble solids, ascorbic acid) (Lu et al., 2012b; Hao et al., 2016), have been found. In most studies to date, researchers have integrated supplemental LED lighting technologies either in greenhouses where no supplemental lighting was formerly present or as additional lighting sources in greenhouses where top artificial lights (e.g., high-pressure sodium lights, HPSs, lamps) were already installed and in operation. Accordingly, this study aims to offer an overview of the recent topic of supplemental LED lighting for greenhouse tomato production through the use of a meta-analysis as a statistical tool to summarize the results of published studies and understand the effectiveness of supplementary LED lighting in influencing the qualitative-quantitative aspects of truss tomatoes. Consequently, the meta-analysis aims to answer the following research question: does supplemental LED lighting enhance the yield and qualitative traits of greenhouse truss tomato production?

3.1.3 Materials and Methods

3.1.3.1 Data collection

Article collection was conducted during the first half of 2020 using online databases (e.g., Google Scholar and Scopus). The following search string was applied to identify publications: LED *AND* supplemental light* *AND* tomato* *AND* greenhouse. Only accessible published material in the English language was collected, including scientific articles, conference papers, book chapters and thesis dissertations. The literature search results were then filtered to reduce heterogeneity in the studies and to include only *Solanum lycopersicum* species cultivated in greenhouses with supplemental LED lighting or supplemental LED lighting combined with HPS lamps. All cultivar types, growing systems and greenhouse typologies were considered. Given that the presence of solar radiation was a requisite of the research question (targeting the effect of supplemental LEDs in greenhouses), studies of indoor cultivation in which only artificial lighting sources (e.g., indoor farming) were applied were not considered in the research. Overhead, intracanopy and bottom lighting supplies were all included, as well as nighttime, end-of-the-day and continuous lighting treatments. Only one case of night-break lighting supply was excluded from the research (Cao et al., 2016). Furthermore, studies reporting evaluations on seedlings or transplants with short treatment periods were also excluded; only mature and productive plants were considered to accomplish the upstream objective of evaluating the qualitative and quantitative effects of supplemental LED lighting on tomato production.

The collected data included both general information related to trial conditions and more specific data used in the meta-analysis. In particular, the general data were represented by intrinsic or environmental trial features (cultivar, location, maximum and minimum temperatures, relative humidity, nutrient solution electrical conductivity (EC) and pH, plant density, greenhouse typology and growing system), as well as by the LED treatment characteristics (light spectrum, intensity and photoperiod, treatment duration and other eventual specific experimental conditions, e.g., nighttime treatments). All these general data were used in the descriptive statistical analysis and to identify factors of heterogeneity among different experiments during the meta-analysis. The natural lighting amount (e.g., DLI) was not considered due to the scarcity of articles reporting this information. The specific data referred to precise information that was needed for the development of the meta-analysis, including the treatment and control mean outcomes as well as the sample size (or replicate number, in cases in which the sample size was not available). Studies not reporting specific data were not used for the meta-analysis development. The outcomes, also called the effect sizes or response ratios [R] (Hedges et al., 1999), used in the meta-analysis consisted of the fresh fruit mass yield (yield, expressed as kg plant^{-1} of fresh fruit mass), soluble solid content (TSS, expressed as °Brix), ascorbic acid content (Asc, expressed as mg Asc 100 g^{-1} of fruit fresh weight), chlorophyll content (Chl, expressed as Chl index), net photosynthesis (PN, expressed as µmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s, expressed as mmol H_2O m^{-2} s^{-1}) and leaf area (LA, expressed as m^2 per plant). Only physiological and vegetative outcomes directly influencing tomato productivity and quality were considered, while other information (e.g., stem diameter, internode length) was not investigated. Outcome values were extracted from both tables and graphs, integrating textual information in cases of general descriptive data relative to the trials. **Figure 10** shows the flow diagram applied for the data selection and evaluation.

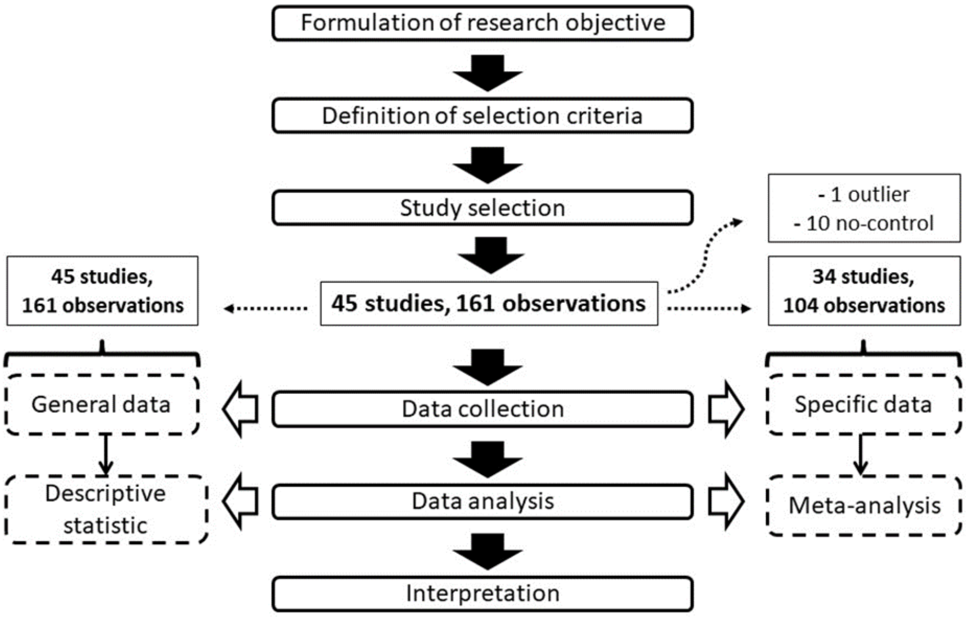


Figure 10. Flow diagram showing the steps of the study selection and analysis.

3.1.3.2 Meta-analysis

The response ratio [R] considered in the meta-analysis was represented by the influence of supplemental LED lighting on the Yield, TSS, Asc, Chl, PN, g_s and LA of greenhouse-grown tomato plants. Since each study accounted for more than one treatment, each trial was examined as a separate observation (k). Accordingly, each study could have more than one observation. For instance, if an article compared two different supplemental LED lighting treatments, one with a Red spectrum and the other with a Blue spectrum, each treatment was considered an individual observation. Each treatment value was compared with a control value from the same article to perform a meta-analysis based on the differences among independent groups. In the current study, the applied control treatment may be of two types: solar light only or solar light + HPS light. In the first case, the control was compared with solar light + supplemental LED light, while in the second case, the comparison involved solar light + HPS light + supplemental LED light. Only observations reporting a control, either with solar or solar + HPS light, were used for the meta-analysis after a second selection phase. In one case (Deram et al., 2014), the comparison between the control and treatment showed extremely high values compared to other results. In this case, the data were considered outliers and were excluded from the meta-analysis.

The [R] of each observation was calculated as follows:

$$\ln \mathrm{R} = \ln (\mathrm{R}) = \frac{\ln \mathrm{m_t}}{\ln \mathrm{m_c}} = \ln \mathrm{m_t} - \ln \mathrm{m_c}$$

where m_t and m_c represent the mean outcomes of the treatment and control, respectively (Hedges et al., 1999; Borenstein et al., 2009). Since most of the considered publications did not display standard error (SE), variance (Var) or standard deviation (S) values, an unweighted meta-analysis was applied to equally weight each observation (McDaniel et al., 2014; Qin et al., 2015). The data were analyzed using the online available software Meta-Essential (Suurmond et al., 2017). A random effect model was chosen for each response value (Yield, TSS, Asc, Chl, PN, g_s and LA). The heterogeneity value (I^2) was used to evaluate the percentage of variation among studies (Hak et al., 2016). Cases reporting I^2 values higher than 25% were further investigated by applying a subgroup analysis (Borenstein et al., 2009; Hak et al., 2016). The subgroup analysis divided the observations into six categories: solar light or solar light + HPS used as a control; pure supplemental LED light or supplemental LED light + HPS; artificial light supply (e.g., DLI ≥10 or <10 mol m^{-2} d^{-1}); seasonality (whether the hours of natural light were increasing, e.g., during spring in the Northern Hemisphere, or decreasing, e.g., during fall in Northern Hemisphere, along the experiment); photoperiod ≥16 or <16 h d^{-1}; lighting supplied as intracanopy or others. In the last case, "others" were intended to include overhead, bottom or combined lighting supplies, which were grouped together due to the low number of singular categories. Hedges' g was applied as the measurement of the effect size in the meta-analysis model. The [R] value was accepted as significant with a p-value ≥0.05, considering a confidence interval (CI) of 95%. Since the results showed high heterogeneity, no publication bias analysis was performed, assuming its absence (Hak et al., 2016). A graphic representation of the study distribution per year and country was realized using Gephi software (Bastian et al., 2009) (**Figure 11**).

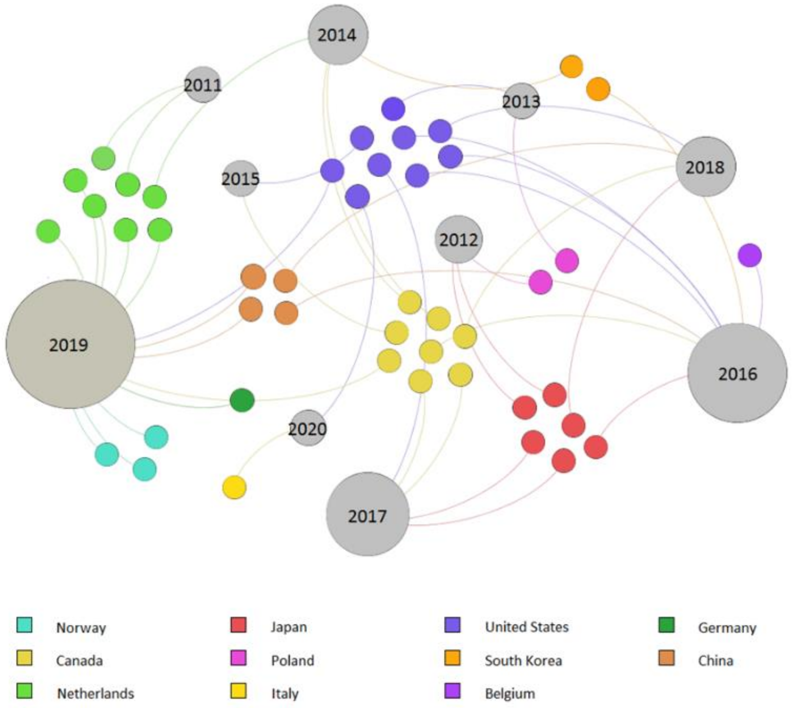


Figure 11. Graphical distribution of 45 selected studies grouped by country and publication.

3.1.4 Results

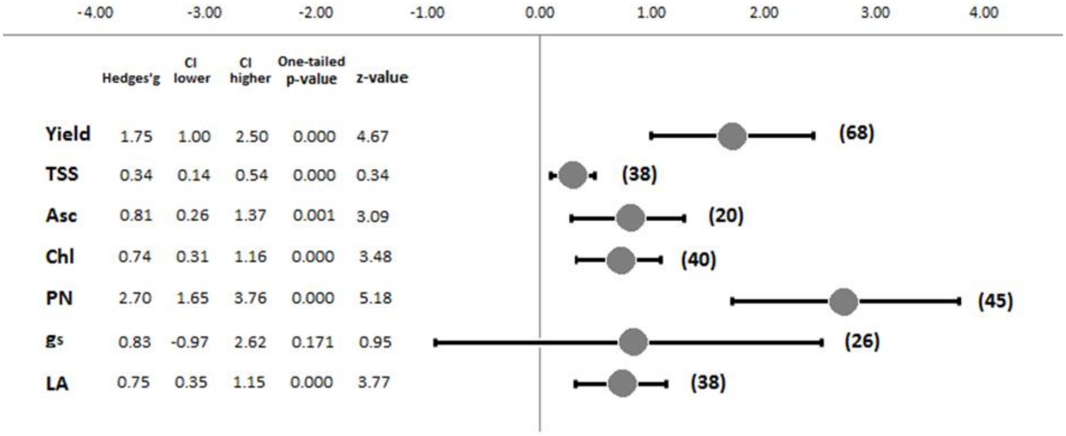
The literature search results are included in Supplementary Materials S1, attached as an Excel file to the present manuscript. The preliminary literature search resulted in 45 studies following the selection criteria. These publications were used for the descriptive statistical analysis. The results showed that the majority of trials took place in North America, with 38% of the total cases (USA n=9, Canada n=8), while Europe (Netherlands n=8, Norway n=3, Poland n=2, Belgium n=1, Germany n=1, and Italy n=1) and Asia (Japan n=6, China n=4, and South Korea n=2) reported frequencies of 35% and 27%, respectively. No cases were registered in other continents. No collected publication was released before 2011, and the collected studies showed the highest frequencies in 2019 (29%) and 2016 (18%) (**Figure 11**).

Although not always stated, most experiments were conducted in technologically advanced greenhouses applying soilless growing methods, and the studies often mentioned controlled environmental systems. When reported, the highest-frequency growing methods reported were substrate cultivation on slabs (61% of 36 cases reported this growing method). Slab materials were mainly represented by rockwool, although 2 cases of coir use were also reported. Pot employment was also registered, occurring for 22% of cited cases with sand, perlite, vermiculite or peat applied as growing substrates. The use of bags filled with substrate (peat, vermiculite or perlite) was also identified in 3 out of 36 cases. Finally, only two soil-based cultivation cases and one nutrient film technique (NFT) case were reported.

Concerning the planting density, the 45 studies showed a mean of approximately 5 plants m^{-2}, a mode of 2.7 plants m^{-2} and a median of 2.7 plants m^{-2}, with values ranging from 1.5 to 16.6 plants m^{-2}. The average maximum and minimum temperatures were 23°C and 19°C, respectively, while the average relative humidity was 69%. The applied nutrient solutions had a mean EC value of 2.4 dS m^{-1} and a mean pH value of 6. Different cultivars of truss tomato were used in the trials (33), and the highest recurrence was observed for *Solanum lycopersicum* cv Komeett (De Ruiter, Amstelveen, The Netherlands), which was mentioned in 10 publications.

In total, 161 supplemental LED lighting treatments were observed within the 45 collected publications. Of those treatments, 57% applied intracanopy LED lighting, 8% applied bottom lighting, 17% applied overhead lamps, 13% applied a combination of supply methods (e.g., intracanopy + overhead lighting), and 6% of cases did not clearly report the type of lighting supply. Furthermore, 20% of revised observations applied a combination of LED and HPS lighting as the supplemental treatment. Regarding the daily lighting duration, the mean photoperiod used was 15 h d^{-1}, while the mode and median durations were both 16 h d^{-1}. Within the collected literature, two extreme cases of 24 h of continuous lighting and 2 h of end-of-the-day lighting were also found. The average photosynthetic photon flux density (PPFD) and DLI supplied through lighting were also registered, showing values of 165 µmol m^{-2} s^{-1} and 9.5 mol m^{-2} d^{-1}, respectively, while the mode and median were 160 and 165 µmol m^{-2} s^{-1} for PPFD and 11.5 and 9.8 mol m^{-2} d^{-1} for DLI, respectively. Spectral compositions occurred in numerous combinations and ratios depending on the trial. The absolute frequencies of the Red, Blue, White, Far-Red and UV spectral components were registered separately, and each component was counted each time it appeared in a treatment independently from the combinations. The count resulted in Red light supplies recurring in 128 cases, while light in the Blue, White and Far-Red spectra were adopted 115, 24 and 20 times, respectively. UV application was only used 3 times, while Green light occurred once. Both UV and Green light were always applied in combination with other light spectral components. Furthermore, 68% of the reviewed observations used a combination of Red and Blue diodes, while monochromatic Red or Blue diodes were applied in 6 and 2% of total observations, respectively. The average duration of treatments was 5 months, with the durations ranging from 2 to 8 months.

After a second selection phase, 10 studies not reporting any control, as well as one outlier case concerning the reported yield values (Deram et al., 2014), were excluded from the meta-analysis. This selection resulted in 34 studies, and 104 total observations were used for the further analyses. The results revealed the generally positive effects of supplemental LED lighting, although different tendencies and significances were observed depending on the evaluated [R] (**Figure 12**). PN (k=45) and Yield (k=68) were the parameters most affected by supplemental LED treatments, reporting the highest standardized mean differences (Hedges' g) of 2.70 and 1.75, respectively. Both response ratios were significantly influenced by supplemental lighting, with one-tailed p-values <0.001. Conversely, g_s (k=26) showed a standardized mean difference of 0.83, although no significant effect was reported (p=0.171). Asc (k=20) and TSS (k=38) presented standardized mean differences of 0.81 and 0.34, with significant p-values of 0.001 and <0.001, respectively. Finally, Chl (k=40) and LA (k=38) recorded similar values, showing Hedges' g values of 0.74 and 0.75, respectively, while the p-values were <0.001 for both cases. **Figure 12** displays a summary of the combined effect sizes.



	Hedges's g	CI lower	CI higher	One-tailed p-value	z-value
Yield	1.75	1.00	2.50	0.000	4.67
TSS	0.34	0.14	0.54	0.000	0.34
Asc	0.81	0.26	1.37	0.001	3.09
Chl	0.74	0.31	1.16	0.000	3.48
PN	2.70	1.65	3.76	0.000	5.18
g_s	0.83	-0.97	2.62	0.171	0.95
LA	0.75	0.35	1.15	0.000	3.77

Figure 12. Forest plot showing the combined effect sizes and main meta-analysis parameters of the investigated response ratios (Yield, *Yield*; soluble solid content, *TSS*; ascorbic acid content, *Asc*; chlorophyll content, *Chl*; photosynthetic capacity, *PN*; stomatal conductance, g_s; leaf area, *LA*). Numbers within brackets refer to *k* response ratios. The meta-analysis parameters are the effect size value (Hedges'g), low and high confidence intervals (CI), and tests of the null hypothesis (one-tailed p-value and z-value) (Hak et al., 2016).

The I^2 value, which describes the percentage of variation among studies, was the main investigated factor used to understand the heterogeneity in the results (Hak et al., 2016). In particular, fruit yield (Yield) showed a heterogeneity of 89.18%. The qualitative effects, measured as TSS and Asc, reported I^2 values of 29.27% and 75.97%, respectively. The physiological parameters showed I^2 values equal to 91.66% for g_s, 91.15% for PN and 73.23% for Chl. Finally, the LA heterogeneity was 80.04%. The other parameters explaining heterogeneity are reported in **Table 3**.

Table 3. Heterogeneity evaluation of response ratios (Yield, *Yield*; soluble solid content, *TSS*; ascorbic acid content, *Asc*; chlorophyll content, *Chl*; photosynthetic capacity, *PN*; stomatal conductance, g_s; and leaf area, *LA*). The heterogeneity parameters are the weighted sum-of-squared differences between the observed effects and the weighted-average effects (Q); the test of the null hypothesis (p_q); the measure of the proportion of observed variance that reflects the real differences in the effect size (I^2); and the measure of the dispersion of the true effect sizes between studies in terms of the scale of the effect size (T^2 and T) (Hak et al., 2016).

	Q	p_q	I^2	T^2	**T**
Yield	619	<0.001	89.18	3.45	1.86
TSS	52.31	0.049	29.27	0.10	0.31
Asc	79.07	<0.001	75.97	0.86	0.93
g_s	299.70	<0.001	91.66	4.07	2.02
PN	497.14	<0.001	91.15	4.18	2.05
Chl	145.67	<0.001	73.23	0.76	0.87
LA	185.33	<0.001	80.04	1.09	1.05

All response ratios showed high heterogeneity, with I^2 values >25%. Accordingly, a subgroup analysis was performed for each outcome. Low heterogeneity was observed for Yield in cases of light supplies different from intracanopy supplies (I^2=18.7%) and cases using solar + HPS lighting as controls (I^2=12.7%); for TSS in cases with increased natural lighting (I^2=21.2%) and lighting supplies other than intracanopy supplies (I^2=0.0%); and for Chl in cases with decreased natural lighting (I^2=0.0%). **Table 4** shows the I^2 heterogeneity values identified for each [R] value and subgroup, as well as the percentages of each subgroup both relative to the single response ratios and to the total number of meta-analysis observations. Cases not reporting a sufficient number of observations ($k \geq 5$) within each subgroup division were not reported.

Table 4. Subgroup analysis reporting heterogeneity (I^2) and percentage (P) by response ratio (Yield, *Yield*; soluble solid content, *TSS*; ascorbic acid content, *Asc*; chlorophyll content, *Chl*; photosynthetic capacity, *PN*; stomatal conductance, g_s; leaf area, *LA*) and total percentage (Tot P) of subgroups considering 104 total observations used in the meta-analysis. I^2 values < 25% are reported in bold.

	Yield (k=68)		**TSS (k=38)**		**Asc (k=20)**		**g_s (k=26)**		**PN (k=45)**		**Chl (k=40)**		**LA (k=38)**		**Tot (k=104)**
	I^2 (%)	P (%)	I^2 (%)	P (%)	I^2 (%)	P (%)	I^2 (%)	P (%)	I^2 (%)	P (%)	I^2 (%)	P (%)	I^2 (%)	P (%)	Tot P (%)
Control type															
Solar light	92.2	69.1	-	97.4	-	85.0	-	100	-	97.8	-	90.0	79.8	86.8	80
Solar + HPS	**12.7**	30.9	-	2.6	-	15.0	-	0.0	-	2.2	-	10.0	77.5	13.2	20
Lamp type															
LED	92.6	64.7	-	94.7	-	80.0	-	92.3	-	93.3	75.1	87.5	78.9	86.8	76
LED+HPS	28.9	35.3	-	5.3	-	20.0	-	7.7	-	6.7	49.9	12.5	67.5	13.2	24
DLI															
<10	76.9	52.9	34.4	44.1	81.6	45.0	88.1	65.4	87.6	55.6	81.1	50.0	72.5	50.0	42
>=10	93.2	47.1	36.7	55.9	71.2	55.0	93.7	34.6	92.2	44.4	56.9	50.0	83.5	50.0	58
Photoperiod															
<16	80.8	35.3	45.5	41.9	84.4	45.0	92.8	34.6	90.3	37.8	88.5	30.0	35.8	34.2	30
>=16	91.1	64.7	28.6	58.1	71.3	55.0	91.4	65.4	90.4	62.2	43.2	70.0	84.9	65.8	70
Natural light															
Decreasing	86.6	30.5	52.9	33.3	84.9	38.9	91.2	28.0	95.8	27.0	**0.0**	16.1	-	11.1	30
Increasing	91.2	69.5	**21.2**	66.7	67.7	61.1	90.4	72.0	83.2	73.0	66.2	83.9	-	88.9	70
Light supply															
Intracanopy	91.9	72.1	36.9	84.2	-	80.0	92.6	62.2	92.9	68.9	73.4	57.5	86.7	57.9	77
Others	**7.4**	27.9	**0.0**	15.8	-	20.0	84.7	30.8	81.6	31.1	71.7	42.5	45.0	42.1	23

3.1.5 Discussions

The worldwide distribution of the 45 identified studies showed a prevalence of trials in countries of the boreal hemisphere occurring at latitudes above 43°N, falling within the temperate climatic zone (Fischer et al., 2012). Geographical latitude is one of the main factors constraining daily solar radiation during the year, thus affecting the minimal light requirements of most horticultural crops (approximately 2.34 kWh m^{-2} d^{-1}, which translates to approximately 8.5 MJ m^{-2} day^{-1}) and, consequently, affecting climatic suitability for greenhouse cultivation (Castilla and Baeza, 2013). Accordingly, supplemental lighting can be particularly appropriate to guarantee better light distributions and longer cultivation spans in high-tech greenhouses in northern countries, although useful applications were also observed within the Mediterranean area (Paucek et al., 2020). Although Mediterranean greenhouse cultivation is mainly characterized by applications of low-tech solutions (Fernández et al., 2018), some examples of technologically advanced high-density greenhouse farms also exist in this region (Meneses and Castilla, 2009; Tuzel and Oztekin, 2014). In these cases, supplemental lighting may be applied to improve off-season production. Indeed, research on the application of supplemental LED lighting in the Mediterranean region has already demonstrated the capability of this technology to improve yields and anticipate the ripening of truss tomatoes during spring and summer (Paucek et al., 2020), although further research should be conducted in the fall season. Furthermore, it is important to consider that Mediterranean greenhouse cultivation can suffer from excessive sun radiation and temperatures during summertime, making external shading a necessary technique to ensure good internal growing conditions (Castilla et al., 2002). However, sunlight screening may also reduce plant photosynthesis, especially in cases of low-cost permanent solutions (e.g., whitewashing) (Garcia et al., 2011). Tewolde et al. (2018) demonstrated the feasibility of supplemental LED inter-lighting on tomato production in cases of shading cover applications, obtaining the same qualitative-quantitative performances as those observed in the naturally lighted control. Although LED use was identified as an effective artificial lighting source for horticultural purposes (Heuvelink and Gonzalez-Real, 2007; Gupta and Agarwal, 2017), research on greenhouse-grown tomato production using supplemental LED lighting seems to be relatively recent, as evidenced by the higher number of studies published during the last five years (**Figure 11**). Nevertheless, earlier studies on seedlings and transplants are also present (Brazaitytė et al., 2009; Suzuki et al., 2009), though they were not considered within this research.

High-tech solutions characterized by the use of soilless cultivation systems, controlled climates and high plant densities were mainly adopted in the evaluated trials. Although not always mentioned, some studies reported high-wire growing methods based on plant lowering, allowing for production throughout several seasons (Kubota et al., 2018). This training system, in association with advanced protected growing technologies, can ensure increased productivity despite flourishing vegetation causing inner canopy shading (Hamamoto and Yamazaki, 2009) and light quality modifications that occur due to both greenhouse cladding materials and shading items (Kittas et al., 1999; Petropoulos et al., 2019). An economic analysis demonstrated that these highly productive systems, together with efficient lighting technologies, can make supplemental lighting more effective for greenhouse tomato production than for the production of other species (Kubota et al., 2016). With reference to both the technical and environmental aspects of trial management, the analysis of the results showed that most supplemental LED lighting studies followed the optimal growth conditions suggested for intensive greenhouse tomato production (Schwarz et al., 2014; Kubota et al., 2018). For instance, rockwool was found to be the most-applied growing substrate, as is commonly observed in greenhouse tomato soilless cultivation systems (Kubota et al., 2018). The environmental growth conditions also followed the recommendations for the fruit production phase, suggesting a mean temperature of 21-18°C, with nutrient solutions featuring ECs of 2.7-4.0 dS m^{-1} and a pH value of 5.8 (OMAFRA, 2001). When a supplemental LED lighting system is adopted, temperature management becomes a key factor. Dueck et al. (2011) observed that tomato plants grown under LED lighting receive less radiative energy than when other lamp typologies (such as HPS lamps) are used, thus requiring more thermal heat during cold seasons to maintain an optimal temperature within the greenhouse. On the other hand, Verheula et al. (2019) pointed out that the addition of supplemental LED inter-lighting to HPS lamps can increase the temperature by 1 to 2°C, leading to increased ventilation requirements for greenhouse production during summer. Furthermore, considering that the lifespans of LED lamps are halved when the working temperature increases by

10°C, a cooling system may also be necessary (Nelson and Bugbee, 2014; Hinov et al., 2019). The average planting density value adopted in the considered studies was higher than the suggested greenhouse standards (2.5 plants m^{-2} for northern Europe) (Kubota et al., 2018), even reaching 16.6 plants m^{-2} in some studies (Song et al., 2016; Johkan et al., 2017). Elevated planting densities may negatively affect light absorption in tomato plants (Sarlikioti et al., 2011), but the use of supplemental lighting can compensate for the lower light availability caused by an increased planting density, also enabling higher annual production compared to systems with lower planting densities (Dorais et al., 1991).

The lighting distribution is a fundamental factor associated with optimizing the effectiveness of supplemental lighting systems. Traditionally, overhead lamps were used in greenhouse production systems, resulting in increased upper leaf interception and intracanopy shading (Gomez et al., 2013). Although an overhead lighting supply may be preferred by growers due to both its easy installation in greenhouses and reduced labor requirements for crop management (Gunnlaugsson and Adalsteinsson, 2005), intracanopy lighting can increase light interception within a canopy, enhance light use efficiency thanks to better lighting distribution and maintain the photosynthetic capacities of lower leaves (Trouwborst et al., 2011). The efficacy of intracanopy lighting on tomato production has already been ascertained by using HPS and fluorescent lamps (Gunnlaugsson and Adalsteinsson, 2005; Lu et al., 2012a), although its feasibility for technological uptake emerged only after the introduction of low-surface-temperature LEDs (Hao et al., 2012; Guo et al., 2016). In our research, the majority of considered trials applied intracanopy LED lighting alone, sometimes combined with overhead HPS lamps. Finally, LEDs can also be applied as below-canopy lighting. Supplemental lighting strategies have been shown to increase photosynthesis both below and within the canopy. However, two studies comparing intracanopy light with below-canopy lighting found that the latter technology can promote CO_2 assimilation and stomatal conductance by providing stable light penetration even at low canopy levels (Song et al., 2016; Johkan et al., 2017).

Deram et al. (2014) observed that the responses of plants to supplemental lighting also depended on the spectral components of the lighting system adopted. Red and Blue wavelengths, alone or combined in different ratios, were mainly used in the studies evaluated in our research. In general, Red light was mostly efficient in enhancing photosynthesis (McCree, 1971; Kaiser et al., 2019a), while Blue light was shown to play an important role in controlling plant morphology, biomass accumulation and stomatal conductance (Ménard et al., 2005; Johkan et al., 2010; Ieperen et al., 2012). Monochromatic lighting may be less effective than a combination of Red and Blue light, since combined Blue light can mitigate the so-called "red light syndrome" (seen with monochromatic Red lighting), which manifests itself in reduced leaf growth and decreased stomatal conductance and photosynthetic capacity (Miao et al., 2019). Lu et al. (2012b) observed the effects of monochromatic supplemental lighting on greenhouse truss tomato plants, showing higher yields in cases of Red light application compared to pure Blue light application. However, good results were also obtained by using White light containing both Red and Blue spectral regions in addition to an abundant presence of Green light, which may favor light penetration within a canopy and be particularly suitable for single-truss growing systems (Lu et al., 2012b). Deram et al. (2014) and Kaiser et al. (2019a) highlighted the effectiveness of Red and Blue combinations for tomato production, suggesting Red:Blue=4 and Red:Blue=1.2-2.4 as optimal ratios for yield improvement, respectively. Kaiser et al. (2019b) also evaluated the partial replacement of Red:Blue LED lights with different percentages of Green light (7, 20 and 39%) in cases of greenhouse tomato production with supplemental artificial lighting. The results showed that the highest studied Green percentage (39%), which was similar to the sunlight spectrum, showed the best effects on plant biomass and yield, suggesting that plants may use sunlight-combined wavelengths more efficiently for growth than other wavelength combinations (Kaiser et al., 2019). The Far-Red wavelength was also investigated by several studies on greenhouse tomato supplemental LED lighting (Pepin et al., 2014; Hao et al., 2015; Hao et al., 2016; Gomez and Mitchell, 2016b; Song et al., 2016; Dzakovich et al., 2017; Fanwoua et al., 2019; Ji et al., 2019; Kalaitzoglou et al., 2019; Kim et al., 2019; Zhang et al., 2019; Kim et al., 2020). The Far-Red ratio, particularly the Red:Far-Red ratio, influences phytochrome regulation and has effects on plant architectural development, flower induction, germination, photosynthetic capacity and nutrition (Demotes-Mainard et al., 2016). Zhang et al. (2019) evaluated the effects of different durations of the Far-Red lighting supply (namely, 0.5, 1.5 or 12 h day^{-1}) on greenhouse tomato cultivation, concluding that even when adopting the lowest supply time, plant stem elongation was stimulated, thus enhancing light penetration within the canopy. Kalaitzoglou et al. (2019) also highlighted similar Far-Red-induced morphological and productive effects on tomato plants, although pointing out the necessity for long-term Far-Red supplies during the day to obtain optimal performances. Furthermore, Far-Red light may also improve the hedonic perception of tomato fruit (Kim et al., 2020), despite the potential reduction of resistance to *Botrytis cinerea* (Ji et al., 2019). Finally, Hao et al. (2018) investigated the effects of UV light on tomato yield and did not confirm any significant increase compared to other wavelengths. It should be noted that UV light is traditionally not considered within photosynthetic active radiation (PAR), although recent studies have also attributed the capacity of UV light to foster photosynthesis and growth in plants, e.g., in basil (Dou et al., 2019). Moreover, Tokuno et al. (2012) demonstrated the effectiveness of supplemental UV LED radiation in reducing phytopathological diseases in greenhouse tomato plants. Further research should also specifically target the effect of UV radiation on inducing secondary metabolite production in greenhouse-grown tomato plants, as already observed in several crops (Schreiner et al., 2014).

In addition to lighting quality, the intensity and photoperiod of lighting are also fundamental aspects. Deram et al. (2014) investigated different supplemental LED lighting intensities (135, 115 and 100 μmol m^{-2} s^{-1}), although no statistically significant differences in plant productivity were observed. However, studies on light intensity are still limited, and further investigations of the optimization of plant photosynthetic responses while minimizing energy costs are needed (Weaver et al., 2019). Concerning the photoperiod, the tomato is a photosensitive species with an optimal photoperiod identified at approximately 14 h d^{-1} (Dorais et al., 1995, Demers and Gosselin, 2000). Continuous lighting (24 h d^{-1}) for 5 to 7 weeks may improve tomato plant growth and tomato yield, while a longer supply period can have negative effects, likely caused by accumulations of sucrose and starch affecting the maximum quantum efficiency of photosystem II (PSII) with consequent leaf chlorosis (Demers and Gosselin, 2000; Velez-Ramirez et al., 2017). However, the alternation of Red and Blue continuous LED lighting was reported to reduce plant injuries, with potential applications for long-term yield improvements (Lanoue et al., 2019). Additionally, the period of the day (daytime or nighttime) in which additional lighting is supplied may affect plant production. Particularly, Tewolde et al. (2016) confronted daytime versus nighttime (applying light from 4:00 am to 4:00 pm in the case of daytime supply and from 10:00 am to 10:00 pm for nighttime treatment) supplemental LED lighting applications, reporting a significant increase in yield, as well as of soluble solids and ascorbic acid, during wintertime in the case of nighttime supply and also observing better cost-effectiveness of nighttime supply compared to diurnal applications.

The meta-analysis results showed that the application of supplemental LED lighting on greenhouse tomato plants has a statistically significant tendency toward enhancing Yield, TSS, Asc, Chl, PN and LA, while no significant results were observed for g_s (**Figure 12**). With reference to Yield, although the tendency revealed a global positive effect (with an average yield increase of +40% from the control conditions), some studies reported negative or equal output values compared with their control treatments. These inconsistencies may be attributed to different trial management aspects and should be considered to obtain the best tomato cultivation performance using supplemental LED lighting. Tewolde et al. (2016) observed that daytime LED inter-lighting during summer may reduce tomato yield compared to a solar light control, probably due to the excessive temperature and radiation around the mid-canopy area caused by lamps. A similar effect was also observed by Verheula et al. (2019), equally pointing out the need for ventilation during summer, although with lower energy savings. Additionally, Gomez (2016) reinforced these observations, concluding that supplemental LED lighting may not be a feasible solution during summer even when a root cooling system is used.

Looking at qualitative parameters, while most of the analyzed studies associated supplemental lighting with positive effects (increasing TSS by 6% and Asc by 11%), some inconsistent results were also found. Accordingly, Dzakovich et al. (2015; 2017) reported that supplemental lighting did not increase the TSS values. Similarly, supplemental lighting on tomato plants was not associated with increased TSS values or Asc contents (Lu et al., 2012b) or with the sugar or acid contents, according to Gautier et al. (2005). However, it is important to consider that in addition to light access, other factors may affect the qualitative parameters of tomato fruits (e.g., genotype, environmental conditions, nutrient solution EC) (Kubota et al., 2012; Dzakovich et al., 2015; Ouzounis et al., 2016). Furthermore, it must be acknowledged that the parameters used for the purpose of this research (e.g., TSS and Asc) do not entirely describe tomato fruit quality from a sensorial or nutraceutical standpoint. For instance, due to the scarcity of studies, some qualitative aspects (e.g., antioxidant content) were not evaluated in the present research. Further research on the antioxidant response to supplemental LED lighting is therefore needed, also considering that a potential increase in carotenoids induced by using Far-Red light has already been reported (Hao et al., 2016).

In this study, the leaf response to supplemental LED lighting was evaluated in terms of Chl, PN, LA and g_s. As already presented within the results (**Figure 12**), the response ratios [R] for Chl, PN and

LA globally reported statistically significant increases when supplemental LED lighting was applied (on average, increasing Chl by 31%, PN by 50% and LA by 9%). Additionally, for these parameters, however, inconsistencies were observed among studies. In particular, Kim et al. (2019) observed reductions in Chl and LA in plants treated with low Red:Far-Red levels for long durations, which may be attributed to major biomass allocations in reproductive structures during plant growth and development. Other authors also observed non-statistically significant differences in both the chlorophyll content and total leaf area (Jiang et al., 2017) or in the leaf area only (Gómez and Mitchell, 2016b) when applying supplemental lighting treatments. No statistically significant effect of supplemental LED lighting application on PN was observed by Gajc-Wolska et al. (2013) or by Gomez (2016) compared to the control conditions. From the meta-analysis, a nonsignificant effect of supplemental lighting on g_s was observed, possibly suggesting that excessive light irradiance could also lead to stomatal closure (O'Carrigan et al., 2014a).

The evaluation of heterogeneity among the studies showed high values for each response ratio [R] (**Table 3**). Such results were, however, expected, considering not only the variability in trial management (e.g., diverse locations and technologies, light qualities, intensities, growing systems, etc.) but also the absence of common meta-data protocols for data collection and presentation. The last can be seen as one of the main issues hindering the development of agricultural meta-analyses, and this challenge should be overcome by always presenting all the statistical values needed for a meta-analysis evaluation (e.g., the standard error, standard deviation, variance, and sample size), as well as by applying common measurement methods (Eagle et al., 2017). The lower heterogeneity observed for TSS than for the other factors may be attributed to different measuring systems or units, while the other evaluated effect sizes utilized different measurement standards that required unit conversions in some cases. Concerning the subgroup analysis, most of the confronted group showed high heterogeneity, indicating the absence of influences determined by specific trial characteristics. However, low heterogeneity was observed for Yield in cases of lighting supplies different from intracanopy lighting or pure HPS lamps used as controls, for TSS in cases of increased natural light or other lighting supplies, and for Chl in cases of decreased natural lighting. Accordingly, the analysis revealed common trends of results in these specific subgroups. However, further targeted assumptions regarding the effect of specific LED lighting features (e.g., decreasing or increasing natural sunlight; intracanopy or other light supplies) on the combined effect sizes cannot be hypothesized due to the absence of homogeneity in the confronted group.

3.1.6 Conclusions

Despite some limitations commonly occurring in agricultural meta-analyses, the research conducted herein revealed that supplemental LED lighting may be effective in improving the quantitative and qualitative aspects of greenhouse-grown truss tomato production. Significant positive results were observed for both direct qualitative-quantitative parameters (Yield, TSS, Asc) and crop photosynthetic properties (Chl, PN, LA), while only stomatal conductance (g_s) was not significantly affected by supplemental LED lighting. Further research is needed regarding product quality, particularly focusing on the unexplored effects of LED lighting on nutraceutical properties and organoleptic features. Moreover, most studies considered herein applied Red and Blue spectra, although preliminary studies have also introduced promising results by applying UV or Green light. Finally, the collected studies were mainly concentrated in the northern part of the boreal hemisphere, where the presence of technologically advanced greenhouses, as well as some favorable environmental conditions due to lower temperatures and sun radiation, have induced the wide uptake of horticultural LED technology. However, interesting applications may also be hypothesized for milder climates such as those of the Mediterranean area, in which supplemental LED lighting could improve the quantitative and qualitative aspects of greenhouse tomato plants both during the off-season and when extremely hot summers occur and intensive shading is needed.

3.2 Chapter 2: The global rise of urban rooftop agriculture: A review of worldwide cases

Elisa Appolloni[1], Francesco Orsini[1], Kathrin Specht[2], Susanne Thomaier[3], Esther Sanyé-Mengual[1], Giuseppina Pennisi[1], Giorgio Gianquinto[1]

[1]University of Bologna Alma Mater Studiorum, Department of Agricultural and Food Sciences, Bologna, Italy

[2]ILS—Research Institute for Regional and Urban Development, 44135, Dortmund, Germany

[3]Technische Universität Berlin, Department for Urban and Regional Economics, Berlin, Germany

3.2.1 Abstract

Rooftop agriculture is a building-based form of urban agriculture that employs underexploited urban spaces on buildings for farming purposes as a useful strategy for targeting global concerns (food availability, land access, climate change and social exclusion). While preliminary reports and analyses have addressed rooftop farming cases and the worldwide dissemination of rooftop agriculture, a global and systematic evaluation integrating the constantly evolving sector and its diversity (both commercial and noncommercial) is currently lacking. Here, we present the current status of rooftop agriculture worldwide with the goal of developing guidelines for future sector advancements based on a compiled database and a metadata analysis of 185 case studies. Results showed that most of the studied rooftop farms and gardens are located in North America (44%), while scarcity of cases is observed in the Global South where applications may help improving food security concerns and incomes creation. Rooftop agriculture practice is mainly represented by open-air experiences (84%), whereas the growing sector of rooftop greenhouses is still relatively small despite the potential capability enhancing crop yield. Similarly, commercial cases are relatively scarce, as improving urban living quality and social-educational farming are the main functions of rooftop agriculture. This tendency suggests a range of currently untapped business opportunities that, if developed, may contribute to the evolution of more sustainable and resilient city food systems providing fresh crops form the inner of urban fabric. In Conclusion, the research showed a rising global interest for rooftop agriculture, although a stronger intervention by policy makers would be definitively crucial to reach such an extension of the practice to guarantee decisive environmental, economic and social benefits on the city context.

Keywords: Urban Agriculture, Urban Sustainability, Urban Planning, Innovation, Urban Farming, Building-integrated Agriculture.

3.2.2 Introduction

After the Rio+20 Conference in 2012, the United Nations instructed governments to create effective policies targeting an ensemble of Sustainable Development Goals (SDGs) to guide the post-2015 development agenda (Griggs et al., 2013). A set of 17 SDGs were defined and subdivided into 169 objectives addressing, among various issues, environmental impacts and their interconnections with poverty and marginalization (Stevens and Kanie, 2016). In this context, the development of a “green economy”, including aspects of circularity and biobased industries, has been identified as a central theme for addressing both economic and environmental crises related to the current global situation, also referred to as the Anthropocene era (Steffen et al., 2007; Bina, 2013). Urban areas play a central role in achieving green growth and sustainable development, as more than half of the global population currently lives in urban areas (FAO-FCIT, 2018), and the evolution of the urban fabric is relevant to the further development of green practices such as urban agriculture (UA) (Mougeot, 2006). In fact, as already observed in North America (Palmer, 2018) and Europe (Lohrberg et al., 2016), farming within or on the fringes of cities may become an innovative practice for improving urban sustainability by promoting ecological, social and economic benefits. Furthermore, urban green and productive areas may fundamentally increase cities' resilience to unexpected events such as the recent Covid-19 pandemic, which affected food purchasing of many urban dwellers, highlighting weaknesses in the current food systems (Lal, 2020). Since 2015, 210 cities worldwide have signed the Milan Urban Food Policy Pact, which supports building more resilient urban food systems by further developing urban and periurban agriculture (Filippini et al., 2019).

Since competing uses and the consequent high costs of land might put a strain on UA development, the exploitation of unused city spaces, such as the rooftops of residential or commercial buildings, may represent a way to overcome development barriers (Gasperi et al., 2016). Plant cultivation on the rooftops of urban buildings – also defined as rooftop agriculture (RA) (Orsini et al., 2017) – has been identified as a functional way to increase ecological services (Oberndorfer et al., 2007; Harada and Whitlow et al., 2020), resilience to climate change (Georgiadis et al., 2017; Gupta and Mehta, 2017) and food availability (Baudoin et al., 2017; Gupta and Mehta, 2017) in cities in addition to contributing to the social and economic inclusion of marginal populations (Van Veenhuizen, 2014; Haase et al., 2017) and those that experience gender inequality (Velmurugan et al., 2019).

RA is a form of building-integrated agriculture (Caplow, 2009; Astee and Kishnani, 2011) or zero-acreage farming (Specht et al., 2014; Thomaier et al., 2015) that includes both protected (rooftop greenhouses) and nonprotected (open-air rooftop gardens or farms) technologies. Based on their main goals, RA projects can be classified into five types: (1) commercial, (2) social-educational, (3) image, (4) innovation or (5) urban living quality (Thomaier et al., 2015). Commercial rooftop farms are usually represented by business-oriented enterprises aiming at profitability. In contrast, social-educational and urban living quality RA projects are often developed without profit aims, concentrating more on the integration of minorities, the education of young people, and the amelioration of living conditions for urban dwellers by offering recreational and community spaces for personal food production. Image RA projects are often associated with hotels and restaurants and mainly use rooftop cultivation for marketing and aesthetic purposes. Finally, innovation RA projects target the research and development of new technology for the improvement of sustainable food production and are mainly built by research centers, universities or start-ups (Thomaier et al., 2015).

Depending on their pursued goals and the local socioeconomic situation, RA projects apply different strategies in terms of the applied growing systems, farm design and management (Viljoen, and Howe, 2012). For instance, while low-technology growing systems normally use inexpensive or recycled materials in order to improve urban food access with a minimum monetary investment (Orsini et al., 2015), business-oriented cases integrate RA into the food market chain using state-of-the-art farming technologies and intensive plant cultivation systems (Specht et al., 2015; Benis and Ferrão, 2018). Such intensive systems commonly apply soilless techniques with inert substrates and hydroponic growing methods to optimize farming inputs and yields and are mainly associated with commercial activities, whereas small-scale kitchen gardens and noncommercial projects often use soil either in raised beds or directly on rooftop surfaces.

Comparing to conventional rural agriculture as well as to ground-based urban farming, RA presents some pros and cons mainly related the distinctive location on top of buildings. Among them, physical feasibility (structural loading, rooftop access), safety and municipal codes (historical constrains, height limitations, fire code), and amplified climate conditions (heavy rains, elevated radiative fluxes and temperature ranges) that occur on rooftops are peculiar challenges that RA has to face (Hui, 2011; Caputo et al., 2017) and that may limit its application and cultivation performance. However, RA can also improve building environmental performance (e.g., by improving thermal insulation or integrating rainwater harvesting systems) and employ building byproducts (e.g., graywater, heat, CO_2 and organic waste) as farming inputs (Sanyé-Mengual et al., 2014; Grard et al., 2015; O'Sullivan et al., 2019), thereby integrating the building and the plant production area (Pons et al., 2015; Sanjuan-Delmás et al., 2018) to reduce the environmental impact of cultivation. Further environmental benefits for city context include biodiversity conservation, water runoff management, air pollution and carbon sequestration, as well as reducing both the urban heat island effect and noise pollution (Van Woert et al., 2005; Takebayashi and Moriyama, 2007; Dunnett and Kingsbury, 2008; Rowe, 2011; McIntyre and Snodgrass, 2017).

Despite demonstrated worldwide interest and the effective productive capacity of rooftop farms (Grewal and Grewal, 2012; Orsini et al., 2014), urban farmers still have to face the challenges that have constrained the adoption of this practice on a larger scale, including high initial costs and uncertain returns of investment, as well as a lack of policies that are supportive to the development of the sector (Delshammar et al., 2017). Similarly, urban dweller perceptions and the low acceptance of nontraditional agricultural systems such as hydroponics also hinder potential RA development (Sanyé-Mengual et al., 2018; Ercilla-Montserrat et al., 2019).

Due to the complexity of RA implementation, there is a need to comprehensively evaluate the current worldwide status in order to examine the potential effect of the urban and climatic context, the distribution of existing RA initiatives, as well as their agronomical characteristics and sustainable practices. Such assessment should focus not only on commercial activities (Buehler and Junge, 2016) but also on noncommercial ones with the aim of better individuating best practices for future sector advancement.

This paper presents a global picture of RA with the aim of developing guidelines for future advances in this sector. To do so, a database of existing RA cases was compiled that includes both commercial and noncommercial (e.g., socially oriented, research) cases. A metadata analysis was also performed to identify weaknesses in and opportunities for RA.

3.2.3 Materials and Methods

The core of this research study is the compiled database of RA cases around the world and the metadata analysis based on the collected information. The database adopts a classification of RA typologies (**Figure 13**) based on growing conditions (protected or nonprotected), cultivation purpose and building characteristics (Thomaier et al., 2015; Buehler and Junge, 2016; Nasr et al., 2017) and including a subdivision depending on whether the structure is devoted to food production only (monofunctional building) or to other uses (multifunctional building) (Buehler and Junge, 2016). The data compiled for each case study included both general and operational aspects. Concerning the general aspects of each case, the data included RA type, building type and farming purpose, as organized and explained in the classification developed by Thomaier et al. (2015) (**Table 5**). Operational aspects were further divided into basic and structural observations (starting and closing date, size, activities performed, typology of organization), agronomical parameters (growing system, type of crop/product, crop yield), resource use (water source, energy source, nutrient typology, nutrient form), social and economic aspects (members, women members, population at risk of social exclusion, costs, income, installation costs) and societal impact (consumers, visits, trainees, users). Sustainability actions were built on the description developed by Buehler and Junge (2016). When data were not available from the examined sources, surveys were sent to rooftop gardens/farm administrators (n=13).

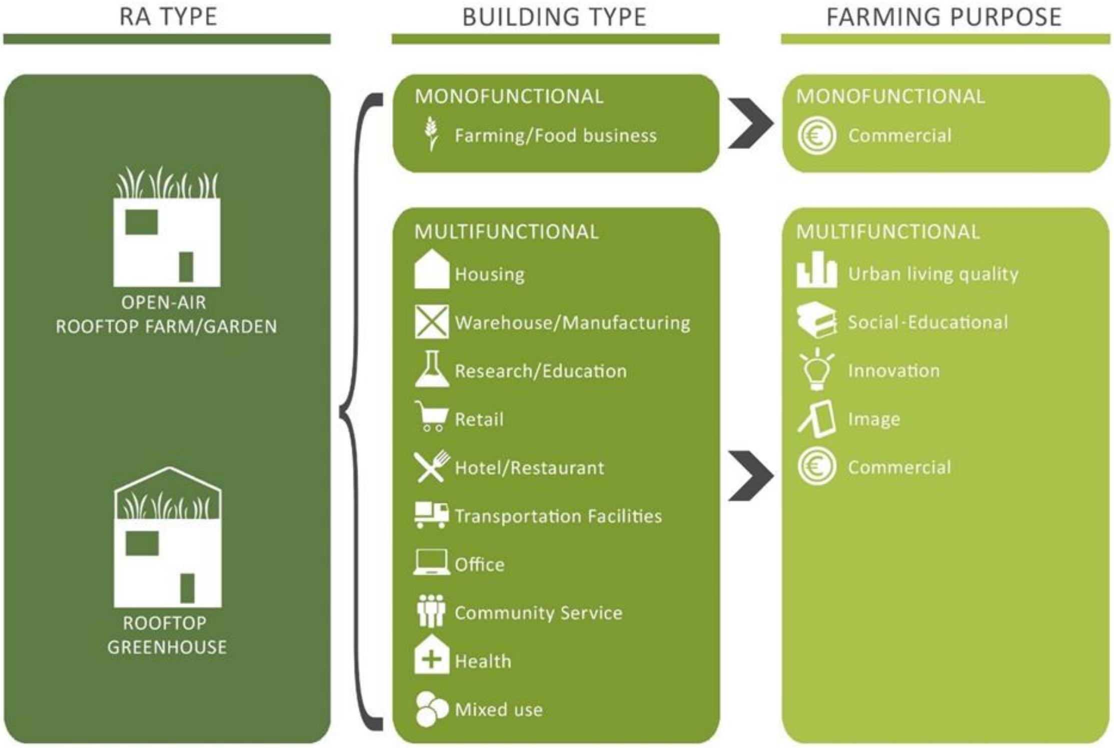


Figure 13. Visualization of case variability with regard to RA type, building type and farming purpose

Table 5. Classification of RA types based on protected or nonprotected cultivation conditions (RA type), building on which RA is located (building type) and farming purpose (Z-farm type, according to Thomaier et al., 2015).

	Subcategory	Description
RA type	*Rooftop Farm/Garden*	Open-air rooftop agriculture
	Rooftop Greenhouse	Protected rooftop agriculture
Building type	*Mixed-use*	Building with different uses
	Housing	Residential building
	Warehouse/Manufacturing	Industrial or storage structure
	Research/Education	University, school, research center, educational center, etc.
	Retail	Retail shop, mall, supermarket, etc.
	Hotel/Restaurant	Hotel, restaurant, cafe, etc.
	Transportation Facility	Train station, bus station, parking lot, etc.
	Office	Bank, post office, company building, etc.
	Community Services	Church, reception or social center, government building, etc.
	Farming/Food Business	Farming-oriented building, possibly integrated with a grocery store or a wholesale shop
	Health	Hospital, clinic, retirement home, gym, etc.
Z-farm type	*Image*	Cases with an image or marketing aim, especially cultivated for the production of food to use in hotel, restaurant and cafeteria kitchens.
	Urban Living Quality	Projects created to improve the living quality conditions of urban residents and employees, offering a green space for producing their own food and recreating (farms or gardens); projects of local or international organization to promote food security and economic development.
	Commercial	Food production businesses
	Social-Educational	Cases often located at schools, hospitals or social centers with educational, social and integration purposes.
	Innovation	Research cases or innovative production systems

The database was built upon an already existing database compiled in 2011-2012 (Thomaier et al., 2015) that was updated and enlarged between 2017 and 2019. The update process consisted of the verification of included cases and their current status, while enlargement phase was performed drawing on two type of sources. The first source type was represented by two recent inventories: the Rooftop Agriculture Handbook by Orsini et al.(2017) and the scientific article by Sanyé-Mengual et al.(2017). The second source type was represented by scientific and gray literature as well as websites, which were explored to identify those RA cases not inventoried in the first type of source. In this case, the search was performed using the following search terms: “rooftop agriculture”, “rooftop farming”, “rooftop garden”, “rooftop greenhouse”, “building-based agriculture”, “zero-acreage farming”, “rooftop aquaponics” and “building agriculture”. Case selection was performed based on strict criteria, and the inventory only included case studies:

1. located on rooftops;

2. reporting basic information about location, RA type, building type and farming purpose;

3. with information available online, either from webpages or secondary reports; and

4. with information available in the English language.

Accordingly, green façades, indoor farms, farming experiences inside shipping containers or other types of structures not located on rooftops, which made up a total of 33 cases, were excluded from the inventory. Since the main objective of the analysis was to understand the global interest in RA and its diffusion worldwide, both ongoing and (potentially) closed cases were included. Reasons for closure were not often declared, although in the case of *UF002 De Schilde* farm in The Hague (Netherlands), closure occurred due to bankruptcy problems, and the *Rooftop Garden of Via Gandusio* in Bologna (Italy) was interrupted due to renovation of the building. Finally, since case searches and data collection were performed in English, language limitations may have biased the resulting database, although a real measurement of the missing cases and information cannot be offered.

The metadata analysis was based on descriptive statistics, mainly including the frequency, mean, mode and median. Descriptive statistics and correlation analysis were applied to the collected data. The descriptive statistics particularly included frequency analyses of the data compiled for each case study, including the RA distribution on each continent and the general and operational data collected. Correlation analysis (Pearson correlation) was performed to identify the existence of relationships (at the 5% level of confidence) at three different levels. At the case study level, we observed a correlation between rooftop surface and rooftop productivity. At the city level, we observed correlations between RA case distributions (frequency by city) and city characteristics (surface, density, and population). At the country level, we observed a correlation between RA case distribution (frequency by country) and the Human Development Index (HDI) (UNDP, 2018). The case distribution by climatic area was also evaluated by classifying cities into five global macro agroecological zones (tropics, subtropics, temperate, boreal and arctic) (Fischer et al., 2012).

Finally, Fisher’s exact tests were used to evaluate at the case study level the association between farm type and farming purpose and the association between farm type and building typology.

3.2.4 Results and discussions

3.2.4.1 Global distribution and trends

The inventory compiled 185 RA case studies from around the world. The distribution of the analyzed RA cases around the world by type (open-air or greenhouse) and farming purpose (image, commercial, urban living quality, innovation or social-educational) as well as the evolution of RA cases in the last 30 years are shown in **Figure 14**.

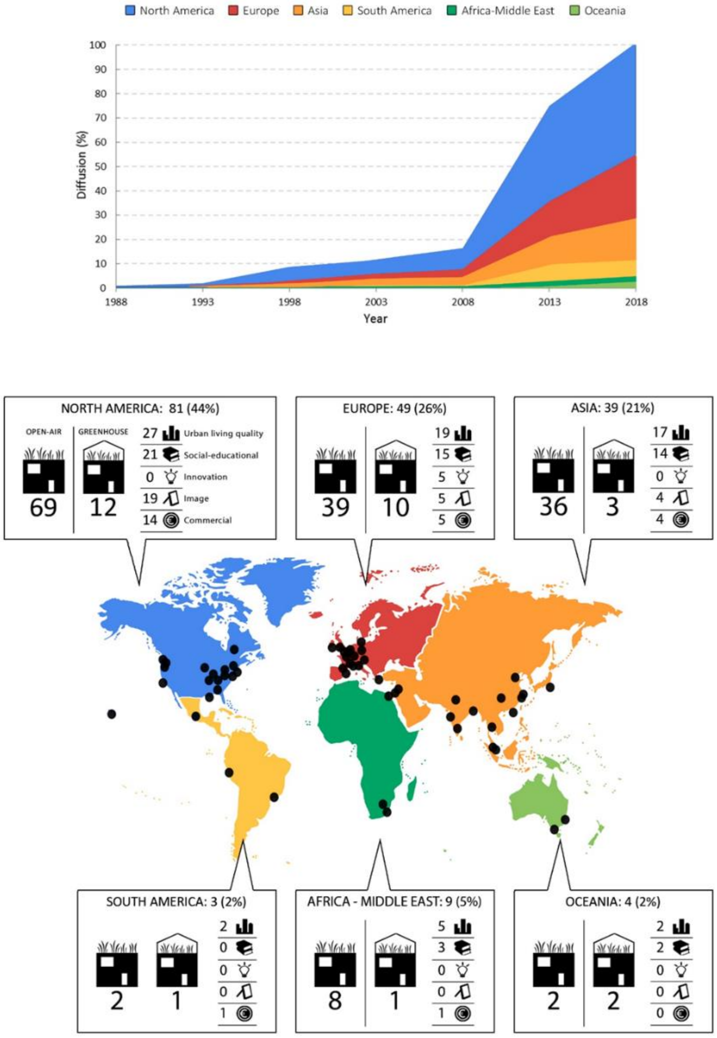


Figure 14. The evolution of RA by continent in the last 30 years (top). The worldwide distribution with a specific focus on world cities with RA projects and the absolute frequency of farming purposes (urban living quality, social-educational, innovation, image, commercial) and RA types (open-air, greenhouse) on each continent (bottom).

According to the analysis of our samples, North America emerged as the continent with the most RA cases (81), followed by Europe (49) and Asia (39). Conversely, Africa-Middle East (9), Oceania (4) and South America (3) presented lower numbers of cases (**Figure 14**). Globally, the trend seems to remain unchanged compared to that in previous research studies (Thomaier et al., 2015; Buehler and Junge, 2016), although new cases have been registered on each continent. Rooftop farms and gardens were mostly identified in the cities of New York (26) and Toronto (15). The overall increase of cases can be interpreted as a rising interest in the practice as a solution to overcome some specific urban issues (Ackerman et al., 2014). However, the reduced number of experiences in the Global South highlights the necessity of further applications also guided by international organization or local policy makers, in order to address food security concerns, small incomes creation and social integration of minorities

No significant correlation was observed between the RA case distribution within cities and the city surface (*r*=-0.044, p=0.71, n=72), city population (r=0.189, p=.112, n=72) or city density (r=0.183, p=0.123, n=72) (data not shown), overall suggesting that RA can easily adapt to different societal needs and challenges. Accordingly, the RA case distribution within a country was not correlated with the HDI (Human Development Index) of the country (r=0.232, p=0.218, n=30) (data not shown).

Classifying cases by climatic zone, the results showed that 69% of RA cases were located in temperate areas, 19% in subtropical areas, and 11% in tropical areas, while no cases were observed in boreal or arctic areas. These results show the variety of climates in which RA can be implemented; furthermore, RA was more widely applied in areas with cooler temperatures despite the greater difficulty of year-round or three-season production. In these cases, RA should apply cold-climate strategies (e.g., protective structures, heating systems) to allow a longer cultivation period.

Rooftop farming can be performed in both nonprotected (open-air farms/gardens) and protected conditions (rooftop greenhouses) (**Table 5**). Protected conditions can help an easier satisfaction of cultivation requirements such as temperature and relative humidity. In fact, an uncovered rooftop can present extreme climatic characteristics comparable to an arid or semi-arid zone, with poor relative humidity and drastic daily and yearly temperature fluctuations (Bazzocchi and Maini, 2017). Although these harsh conditions can require higher watering inputs, as well as eventual shading supports (e.g., shading net), lowering of chemicals use can be obtained thanks to the hostile climatic characteristics against pest development, especially in case of fungi (Bazzocchi and Maini, 2017).

From a global standpoint, the frequency of open-air rooftop farms (156 RA projects, 84%) was 5-fold higher than the frequency of rooftop greenhouses (29 RA projects, 16%). Regarding rooftop greenhouses, although North America had the highest absolute number (12 projects, 15% of RA projects in the region), Europe had the highest relative frequency by continent (20% of RA projects in Europe are rooftop greenhouses) (**Figure 14**). A limited number of rooftop greenhouses were found in Asia (3), Oceania (2), Africa-Middle East (1) and South America (1) (**Figure 14**), possibly because the observed RA projects mainly targeted recreational and noncommercial purposes normally applying open-air agriculture. Regarding the farming purposes (**Table 5**), RA for urban living quality improvement emerged as the most common objective globally (72 RA projects, 39%) and was similarly distributed across different world regions (**Figure 14**). On the other hand, cultivation for sector innovation was documented only in Europe and was therefore the least common farming purpose (5 RA cases, 3%) (**Figure 14**).

Fisher's exact tests showed a statistically significant association between farm type and farming purpose ($p \leq 0.001$). Interestingly, image, social-educational and urban living quality purposes were generally linked with open-air rooftop farms and gardens, while the innovation purpose was more common in rooftop greenhouses (only 1 open-air case was identified out of 5 detected cases). Commercial farms that were intended as food production businesses showed a balance between cases conducted in protected and nonprotected conditions (13 open-air and 13 rooftop greenhouse cases) (**Table 5**), therefore confirming the possibility of running commercially oriented farms with both models; however, there are some differences between the models related to product variability, yield capacity, adaptability to market demand and labor costs (Buehler and Junge, 2016).

Regarding the evolution of RA (n=104), the first examples of rooftop farming cases appeared in the late 1980s and persisted at lower numbers during the 1990s and 2000s. A peak in new rooftop farming cases was noted in 2010, particularly concentrated in North America (**Figure 14**). The growing trend progressively stabilized in the following years, possibly as a consequence of the slowly developing policies in the sector. Nonetheless, it is important to note that most existing cases are still operating, as determined based on updates on their websites. Indeed, only 8 out of 185 cases were officially considered closed, of which 1 was commercial, 3 were social-educational and 4 were urban living quality oriented.

3.2.4.2 Rooftop farms and gardens dimension

Of the 185 case studies analyzed, only 105 cases reported their farming area (data not shown), revealing a global median surface of 600 m^2; the farming areas ranged from a minimum of 4 m^2 to a maximum of 35000 m^2 in the case of a public garden on top of a train station in Paris (*Jardin Atlantique Montparnasse*). The median dimensions of projects were above the global median dimensions in both Europe (750 m^2, n=24, SE=1225) and North America (750 m^2, n=46, SE=257), while the Asian cases had a lower median size (370 m^2, n=19, SE=1867). Lower median surface areas were observed in both Africa-Middle East (20 m^2, n=5, SE=884) and Oceania (130 m^2, n=3, SE=521), which may be attributed to the family-based and residential nature of the cases detected. Concerning the relationship between RA size and type, the median size of rooftop greenhouses was 1390 m^2 (n=25, SE=526), which was larger than the 500 m^2 of open-air RA projects (n=80, SE=640). This difference in size may be attributed to the different main purposes addressed, as rooftop greenhouses are usually applied for businesses and therefore require larger surfaces to achieve economic viability. However, while rooftop greenhouses never exceeded 1 hectare, four open-air RA cases reported dimensions equal to or greater than 1 hectare. Of those open-air cases, all of which were located on large surfaces such the roofs of warehouses/manufacturing buildings or transportation facilities, three were located in Asia, and one was located in Europe. In terms of the relationship between the farmed surface and the farming purpose, RA projects for commercial purposes had the highest median surface area (1860 m^2, n=21, SE=426), followed by projects for urban living quality improvement (560 m^2, n=43, SE=1152), and social-educational aims (500 m^2, n=27, SE=420), respectively. Projects for innovation had a median surface area of 250 m^2 (n=3, SE=2583), while cases for image purpose had a median area of 280 m^2 (n=11, SE=234). Regarding the relationship between case size and growing method (n=71), soilless systems (n=24) had a median surface area that was similar to that of soil-based (soil and organic substrate) systems (n=47), i.e., 555 m^2 (SE=394) and 500 m^2 (SE=264), respectively.

3.2.4.3 Building typologies for Rooftop Agriculture application

RA can be integrated with both new and existing buildings (Caputo et al., 2017). *Maison Productive* in Montreal and *Louis Nine House* in New York are examples of sustainable and affordable housing projects that incorporated rooftop gardens from the beginning into their architectural plans. However, the integration of RA in new buildings accounts for only a limited number of cases; the retrofitting of existing rooftop structures is the more common situation.

Figure 15 displays the absolute distribution of building types by RA types and farming purposes using the classification developed by Thomaier et al. (2015). Fisher's exact tests showed a statistically significant association between farming purpose and building typology ($p \leq 0.005$). Accordingly, structures oriented toward research and education (e.g., schools and universities), as well as residential buildings, were the most common types of constructions used for RA development, accounting for approximately 30% of the total cases (**Figure 15**). On the other hand, buildings entirely oriented toward farming or food businesses that also integrate food production within the building were rarer (2%), although they presented the highest relative frequency of rooftop greenhouses together with warehouses and manufacturing structures (**Figure15**). Predictably, buildings intended for farming and food businesses presented only a commercial purpose, while housing buildings hosting an RA project specifically targeted urban living quality (**Figure 15**). Social-educational purposes were especially common in research and educational centers (54%) (**Figure 15**). Eighty-five percent of the RA projects on hotels and restaurants were devoted to image improvement (**Figure 15**).

RA TYPE		BUILDING TYPE	FARMING PURPOSE				
OPEN-AIR	GREENHOUSE		IMAGE	URBAN LIVING QUALITY	COMMERCIAL	SOCIAL-EDUCATIONAL	INNOVATION
1	3	FARMING / FOOD BUSINESS	0	0	4	0	0
28	4	HOUSING	1	28	0	3	0
7	5	WAREHOUSE / MANUFACTURING	2	4	3	2	1
28	6	RESEARCH/EDUCATION	0	1	0	30	3
9	4	RETAIL	3	5	3	2	0
19	1	HOTEL / RESTAURANT	17	1	1	1	0
7	2	TRANSPORTATION FACILITIES	0	7	1	0	1
18	2	OFFICE	4	8	6	3	0
13	0	COMMUNITY SERVICES	1	6	0	6	0
11	0	HEALTH	0	3	0	8	0
15	2	MIXED-USE	1	9	6	0	0

Figure 15. The absolute distribution of building types by RA type and farming purpose (n=185).

It is important to note that buildings oriented toward farming and food businesses, such as *Ecco Jäger Farm* in Bad Ragaz (Switzerland) or *Toit Tout Vert* in Paris (France), employed not only the rooftop surface but also the indoor building area for food production and were therefore classified as monofunctional buildings that were entirely dedicated to that business. In contrast, other building typologies applied rooftop cultivation on buildings with other primary functions, such as retailing, manufacturing, housing or education, and were therefore classified as structures with multifunctional purposes (Buehler and Junge, 2016). See **Table 5** for further specifications on building typologies.

3.2.4.4 Growing systems typologies

Among the 92 cases that reported data on their growing system, those growing plants on soil (54%) were the most common, followed by RA cases operating on soilless media (33%) and cases using an organic substrate derived from organic matter (e.g., peat, compost) (13%) (data not shown).

Cultivation in soil was performed with either filled raised beds or the direct application of soil on roof surfaces. In the case of the direct application of soil, specific green-roof technologies using roof insulation, drainage systems and low-weight substrates have been used to reduce roof load (Caputo et al., 2017), as observed in the case of *Ortalto – Le Fonderie Ozanam* in Turin (Italy). Among the soilless systems, the cases analyzed reported the use of hydroponic technologies (15), aquaponic technologies (9) and aeroponic technologies (2), and these growing systems were mostly used in rooftop greenhouses (66%, n=20). In contrast, open-air projects (n=70) mostly used soil-based cultivation systems (70%, n=49).

Soilless cultivation was largely applied for commercial (n=15) and urban living quality improvement (n=7) purposes. Specifically, approximately two-thirds of commercial farms used soilless growing techniques; this may be related to the high productivity that hydroponics can achieve, especially when applied in combination with rooftop greenhouses (Buehler and Junge, 2016). Furthermore, soil-based RA projects were mostly connected with social-educational (n=17) and urban living quality improvement (n=16) purposes.

3.2.4.5 Rooftop farming activities and products

Table 6 provides the absolute frequencies of and share of cases performing certain activities in RA projects. Excluding vegetable production (87%), most of the activities performed were linked with education (37%) and recreation (34%). Accordingly, one of the main roles of RA is the opportunity for urban residents to ameliorate their living conditions by exploiting green rooftop spaces for horticultural and gardening workshops, yoga classes, art seminars or relaxation as an escape from the chaotic urban environment.

Table 6. Absolute frequency and share of case studies that perform specific activities. Note that each case study may perform multiple activities (n=152).

Rooftop agriculture activity	Absolute frequency	Share (%)
Vegetable production	133	87
Education	56	37
Recreational space	52	34
Restaurant or bar	21	14
Direct sales of products	20	13
Agricultural training	16	10
Beekeeping	14	9
Distribution of products	12	8
Animal production	10	7
Production of added-value products	9	6
Planning and design services	9	6
Food-related training	8	5
Event rental	7	5

Regarding organization typology, **Figure 16** provides the case distribution by RA type, continent and farming purpose. Of the total number of cases that reported their organization typology (n=145), for-profit initiatives accounted for the highest share (47%). This variable includes not only commercial farms oriented to food production and selling as their main purpose but also other business models with different principal aims (i.e., image, innovation, social-educational, urban living quality). These businesses were hotels, restaurants, RA planning and design consultancies (e.g., *Topager* in Paris, *SUFCo* in Seattle), producers of innovative technologies for rooftop cultivation (e.g., *EFC Systems* and *Zinco Company* in Germany), and event, workshop and tour organizers. It appears that RA may support highly diversified and multifunctional business models and could become an interesting professional opportunity for different types of urban entrepreneurs. It is also important to note that in some cases, the companies involved were real estate agencies or architecture studios that aimed to promote affordable and sustainable housing (e.g., *Banyan Street Manor Rooftop Farm* in Honolulu, *Louis Nine House* in New York, *Maison Productive* in Montreal).

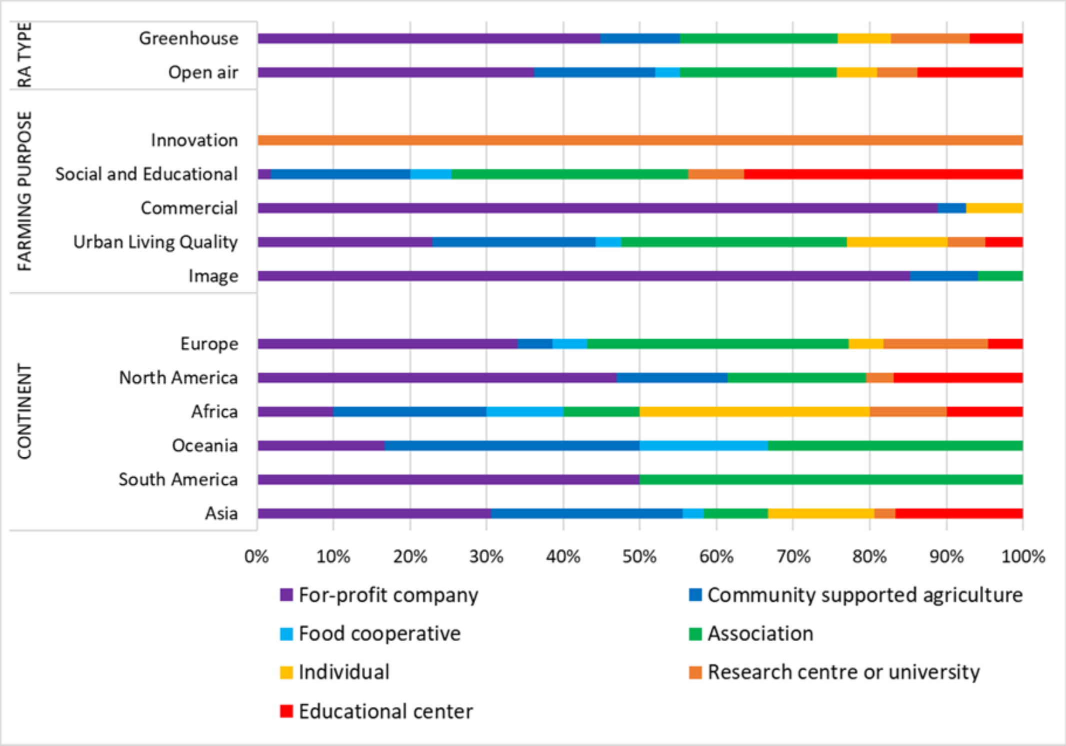


Figure 16. Relative distribution of organization types depending on RA type, farming purpose and continent.

The most commonly produced products were lettuce and herbs (49% and 72%, respectively, of 102 cases), both in open-air and protected systems (data not shown). While soil-based cases normally produced a higher variety of vegetables, soilless systems were usually used to grow herbs, leafy greens or tomatoes. Animal-based products were also reported and mainly included fish (n=8), honey (n=14) and eggs (n=5). Aquaponics was mostly applied in commercial cases (e.g., *Ecco Jäger Farm* in Bad Ragaz, *Comcrop* in Singapore), although one case of private fish production was also registered in the Gaza strip. One unique example of RA use is the production of spirulina, a nutritive microalgae that can be applied as an integrator in different types of products (e.g., pasta, ice cream, chocolate), produced by the *EnerGaia* team in Bangkok.

For 28 cases reporting their productive capacity, the average crop yield was approximately 15 kg m^{-2} $year^{-1}$ (data not shown), overall resembling commercial farming productivity in vegetable crop production, e.g., in the Mediterranean (Orsini et al., 2014). Among those, 11 cases were rooftop greenhouses (10 out of 11 using soilless systems) with an average yield of 28 kg m^{-2} $year^{-1}$, while 17 cases were open-air (2 out of 17 using soilless systems) with an average yield of 6 kg m^{-2} $year^{-1}$. It also emerged that the average yield in soilless systems (30 kg m^{-2} $year^{-1}$) was much higher than the reported yield in soil-based gardens (4 kg m^{-2} $year^{-1}$).

3.2.4.6 Resources and sustainability actions

Table 7 shows the resources applied for crop cultivation, including absolute frequency and share for each type of input. Some cases used more than one type of input. Rainwater was the most common irrigation source, followed by graywater, well water and tap water (**Table 7**). However, due to the limited number of cases that reported their water source, as well as farmers' tendency to report and highlight virtuous actions for environmental preservation, these data should be confirmed through further investigation. The most commonly used energy source was on-grid electricity, although solar panels were also widely applied (**Table 7**). Wind turbines appeared only once in association with solar energy, in the case of the *Gotham Greens Pullman Farm* in Chicago. In some cases, energy was not used or used in negligible amounts for irrigation purposes. Therefore, for the sake of this publication, only cases where energy use was clearly stated were considered. Organic fertilization, generally in the form of compost, was the most common form of nutrient supply (**Table 7**). As mentioned above, further investigations should examine a wider number of cases to better report farmers' practices.

Table 7. Absolute frequency and share of case studies using specific water, energy and nutrient resources.

Resource		Absolute frequency (n)	Share of case studies (%)
Water source		**40**	**100**
	Well water	1	3
	Tap water	13	33
	Rainwater	25	63
	Graywater	3	8
Energy source		**26**	**100**
	Electricity grid	14	54
	Solar energy panel	12	46
	Wind turbine	1	4
Nutrient type		**39**	**100**
	Mineral	12	31
	Organic	17	44
	Compost	24	62
Nutrient form		**33**	**100**
	Solid	22	56
	Liquid	13	33

Applied sustainability actions were also investigated. Among the 79 cases that clearly stated their sustainable management practices, the highest proportion of cases were committed to chemical-free crop production (66%), and this practice was distributed among independent of farming purpose and building type. These cases included both soil and soilless systems, even in countries where soilless systems are not eligible for organic certification. The attention given to chemical-free crop production may be attributed only partially to the necessity of rooftop farmers reducing economic costs; the main reason may be the growing public concern about the use of chemicals in food production and the increasing demand for organic food. As a consequence, reusing recycled nutrients, especially those from compost, was also common; this practice addresses the issue of residual biomass management and favors a circular economy (Manríquez-Altamirano et al., 2020). Technology that improves energy efficiency (e.g., supplementary LED lighting, highly insulating glass) was mainly associated with commercial purposes and rooftop greenhouses and was applied to improve crop yields and reduce production costs. Waste heat reuse (10%), gas exchange (5%) and graywater recycling (4%) were the least-applied sustainable practices, although integrating these techniques into a rooftop greenhouse may help achieving savings of 128 kWh/m^2 of energy and 45.6 kg of CO_2 eq/m^2 (Muñoz-Liesa et al., 2020). Predictably, water reuse was particularly common in projects that used soilless systems; it has been demonstrated that a closed-loop system in a soilless rooftop greenhouse can use 40% less irrigation water and 35-54% less nutrients per day than an open-loop system rooftop greenhouse (Rufí-Salís et al., 2020).

3.2.4.7 Economic and social implications

The economic impact evaluation demonstrated an average installation cost of 880 € m^{-2} for 23 considered cases (data not shown). Installation cost is one of the main constrains that may dissuade from the realization of a rooftop farm or garden. In fact, comparing to ground level cultivation, a rooftop farm has also to consider the costs for the movement of cultivation materials and structures on top of the building, as well as eventual adaptation interventions to guarantee the safety of users and visitors (roof structure reinforcement, safety barriers, emergency exit). However, installation costs may widely vary depending on cultivation purposes and farm typology (open-air or greenhouse), ranging from inexpensive outdoor household experiences obtained with recycled materials to high-tech greenhouses. In fact, commercial and innovation farms usually had higher economic costs than urban living quality and social and educational farms, in which investment costs were probably limited due to the unpredictable economic returns. For instance, commercial cases such as *Comcrop* in Singapore or *Gotham Greens* in Chicago, showed an average installation cost of approximately 1000 € m^{-2}, while that of urban living quality cases such as *Garden City Farmers* in Bengaluru or *Risc's Roof Garden* in Reading, was approximately 300 € m^{-2}.

The running costs and net incomes could be evaluated only for 9 cases, showing 80 € m^{-2} $year^{-1}$ and 26 € m^{-2} $year^{-1}$, respectively, on average. In this case, empirical observations showed that the economic impact could widely very also among the same farming purpose. For instance, the case study of *Ortoalto Ozanam* in Turin, an open-air rooftop garden with social and educational aim, had a running cost of 50 € m^{-2} $year^{-1}$ and an income of 20 € m^{-2} $year^{-1}$. On the other hand, another example of social-educational open-air farm at *NIST International School* in Bangkok presented a sixth of the running costs a tenth of the income. Although this large difference may be imputed to countries purchasing powers, variations ca also be determined by different management conditions (e.g., composting of organic wastes for fertilization, collection of rainwater for watering) impacting on running costs, as well as to incomes coming not only from crops selling but also from the offer of services such as workshops or renting for events. Unfortunately, drawing further conclusions on the economic performance of rooftop farming cases may be difficult due to the limited sample size that reported economic data.

The societal impact of RA was also examined based on the number of people engaged in RA not only as consumers but also as volunteers, trainees or recreational users. A notable case of community involvement was *Schieblock DakAkker* in Rotterdam, which reaches approximately 15000 consumers and 20000 visitors per year. In the Global South, particularly in Africa-Middle East and South America, the feasibility of applying RA to projects involving government and nongovernmental bodies that address poverty and food insecurity has been demonstrated. The potential to involve women in RA was also observed, as in the case of small hydroponic systems in El Salvador (Lima, Perù) that were implemented to improve employment opportunities for women. The involvement of students and children was another important social aspect of RA detected both in the Global South and Global North (e.g., *Rosary High School* in Mumbai, *NIST International School* in Bangkok, *Manhattan School for Children* in New York), with 23 educational centers engaged in rooftop farming projects. Furthermore, the RA projects on top of 8 hospitals and clinics showed an additional social role of RA as a therapeutic treatment for patients. Due to the limited amount of available societal data, however, it was not possible to make deeper quantitative observations.

3.2.4.8 Overall picture of rooftop farming

The analysis confirmed the trends of the increasing geographical distribution of RA around the world and the predominance of certain RA types; there were more RA cases in countries in the Global North and in the form of open-air rooftop farms/gardens. Although a previous study[24] revealed greater interest in social-educational and image-oriented farming purposes, the current investigation showed a wider number of cases intended to improve urban living conditions. However, the hereby study outlined the multifunctionality of RA, as most cases presented a secondary farming purpose, usually combining urban living quality with social-educational or image improvement. At the continent and country level, the presence and distribution of RA cases showed strong variations. In North America, rooftop farming projects are mainly found in larger cities where innovation is promoted through specific policies, such as New York and Toronto. The presence of a high number of cases can be linked to the substantial importance that municipalities in North America place on developing food system strategies and plans to overcome food uncertainty in the urban context (Sonnino, 2016). The main farming objectives are often related to urban dwellers' quality of life or social and educational actions connected to school projects or community integration initiatives. These kinds of projects are normally developed as open-air farming systems, although a few examples, such as the *Manhattan School for Children* in New York or *Concordia Greenhouse* in Montreal, use greenhouses. For-profit companies, including those with commercial and image-oriented farming purposes, are also common in North America, where these RA types occurred at a higher frequency than in other world regions. In terms of commercial farms, North America is also the location of some of the best-known RA food businesses in the world, such as *Gotham Greens* in the US and *Lufa Farms* in Canada. These types of farms are usually rooftop greenhouses, although open-air commercial rooftop farms may also occur, as in the case of *Brooklyn Grange* in New York or *McCormick Place* in Chicago. Although North America showed a higher interest in RA than other regions, cases with RA for innovative purposes were lacking. In contrast, Europe was the only continent promoting innovation in RA at both the academic and private for-profit levels. *AgroParisTech* in Paris and *RTG-Lab* in Barcelona are two examples of European research centers investing in rooftop farming development, while companies such as *UrbanFarmers* in Switzerland and *ECF Systems* in Germany have already developed innovative rooftop aquaponic systems for commercial food production.

Although they are less numerous than in North America, some examples of commercial rooftop greenhouses were also found in Europe, while there were no open-air farms with a commercial purpose as their primary goal in Europe. However, the economic sustainability of commercial rooftop farming is still questioned by European investors; new RA projects such as *Toit Tout Vert* in Paris are opening, but other cases, such as *UF002 De Schilde farm* in The Hague (Netherlands), have had to declare bankruptcy. The latter opened in 2016 and officially closed in 2018. The main reasons for its failure were misunderstandings of both customers and competitors (i.e., due to low receptivity and the high selling price of the products) and the location of the farm in one of the poorest neighborhoods in the Netherlands, far from environmentally conscious and interested customers (Ancion et al., 2019).

The difference in the number of rooftop farming cases in Europe and North America was still notable; there were approximately twice as many North American cases as European cases. This discrepancy may be connected to the slight delay in the increase in European rooftop farming cases compared to that in American cases, as shown in **Figure 14**, as well as other reasons. Due to the large city sizes and strong interest in an organic and safe food supply in Asian countries such as Japan, China and Hong Kong, a higher number of examples from these countries was expected, especially for commercial rooftop greenhouses. The low number in the results may be ascribed to unpublished information or language limitations and should therefore be investigated by native speakers. However, urban living quality and socially-oriented cases turned out to be quite common in Asia in both wealthy countries and less wealthy countries, where this form of agriculture was often employed as a tool to address food insecurity among low-income families. The *Fringe Club Rooftop Republic* in Hong Kong, *Ebisu Garden East Japan Railway* in Tokyo, *Urban Leaves* in Mumbai and the *NIST International School Rooftop Farm* in Bangkok are just some examples of farming projects with social and life quality aims.

Africa and the Middle East had a particularly high proportion of urban living quality and social-educational cases; these were often promoted by local authorities and NGOs, as in some cases of private farming projects established in Egypt, Palestine and Jordan. However, one commercial rooftop greenhouse was registered in Israel.

In South America, RA projects were devoted mostly to social goals to address family food insecurity and urban poverty. Nonetheless, two examples of small-scale hydroponic rooftop farms with commercial purposes were registered in Lima (Perù) and Toluca (Mexico), demonstrating that even under less-advantageous conditions, rooftop farming can be used for business development. Oceania had a very low number of cases compared to those in other continents of the Global North, which some authors attribute to local restrictions on the productive use of rooftops (De Zeeuw et al., 2017).

As previously anticipated, the absence of statistical correlations between the geographical distribution of RA and the size, population and density of cities or HDI suggests that RA is widely applicable for different purposes independent of contextual conditions. However, it is important to highlight that the correlations between the RA case distribution within a city and the city surface area, density and population were not evaluated for all cases due to the difficulty of obtaining comprehensive data from each site and city.

Regarding RA production management, the results of this study are aligned with those in the literature (Buehler and Junge, 2016). Noncommercial cases typically used soil-based open-air systems, which can offer a wider variety of products than protected soilless systems but produce lower crop yields. Conversely, commercial farms preferred soilless systems and greenhouse facilities to maintain a higher production capacity and often focused on specific products such as leafy greens, herbs and tomatoes. The average crop yields of soilless and soil-based systems in UA have been previously studied in published research (Grewal and Grewal, 2012; Orsini et al., 2014; Boneta et al., 2019) that obtained productivity figures similar to or lower than the values presented in this study. This suggests that RA may play a key role within UA in enhancing urban food security. However, the integration of sustainable practices in RA is still limited, specifically regarding technological advancements that integrate plant production with the building metabolism and its byproducts (e.g., heat, gas and graywater), which could increase commercial rooftop greenhouse sustainability.

Particular attention should be paid to the main activities performed on RA farms and the types of organizations that promote RA; these aspects are hardly addressed in the existing literature. Most RA projects provided functions to citizens beyond food production that were combined with a variety of associated services, including event space rentals, rooftop farm/garden planning and design, farming training courses and garden tours. This multifunctionality responds to the increasing need for and awareness of the environment and nature among city dwellers and suggests that RA may offer a wide range of business opportunities for urban entrepreneurs.

The results were gathered mostly from the existing scientific literature and from RA project websites (including surveys of administrators). Accordingly, the number of worldwide rooftop farming cases is certainly higher than that in this study, especially in the case of low-technology projects that do not have a website presence or links with academia. The large amount of data collected per case study in the database led to missing information in specific fields, such as activities performed or organization type, due to incomplete websites or vague information. Moreover, the data were too limited to perform a full social and economic evaluation, and a deeper investigation is required in order to provide a better understanding.

3.2.4.9 Guidelines for future development

RA represents a complementary solution to ground-based and indoor UA, ensuring similar multifunctional benefits while avoiding competition for land access. Despite the established role of environmental, social and economic sustainability in urban contexts, some potential benefits of RA are still underrated. In fact, while RA projects designed for social and recreational purposes seem to have a broad range of applications, the intensive food production capacity of RA is still limited, highlighting its inability to meet current food and nutritional needs in cities. Regulating RA practices, including the agreements between building owners and rooftop farmers, may represent a fundamental step in resolving eventual conflicts and implementing RA projects. Moreover, the recognition of a certification program to ensure product quality and safety, such as certification for chemical-free production or the absence of heavy metals, may also be a key factor in enhancing consumer acceptance and preventing health risks. Similarly, environmental benefits and sustainable practices used in RA could also be included in certification schemes, such as sustainable urban resource use (e.g., rainwater harvesting), building byproduct reuse, urban environmental management (e.g., heavy rain management) or carbon footprint reduction. RA implementation should specifically address the regulatory framework from both the building and farming standpoints, which will require an effort from policy makers to fill legislative gaps and fully develop specific UA regulatory codes. Legislators should consider local conditions and constraints and adapt norms through casuistry to address specific issues. In addition to the need for specific regulations, RA is already shaped by municipal planning codes. Zoning and historical constraints may limit building height and floor number, while safety codes may hinder rooftop accessibility and structural loads. In the latter cases, existing buildings could overcome limitations by adapting the farm design to the circumstances, e.g., using soilless systems to reduce roof loads or installing safety barriers. Because rooftop retrofitting presents limitations, RA should be considered in the design of new buildings in order to integrate food production spaces into the urban fabric to more effectively plan urban food supplies for the future.

Although it is difficult to precisely predict its future development, RA may certainly take on a fundamental role in future cities. Building on its multiple functions, RA may become a strategy for targeting urban issues at different levels, including heat island mitigation, storm water management, biodiversity improvement, social inclusion, food desert and urban poverty reduction, and health and nutrition advancement. However, given the need to optimize resource use efficiency and define economically and environmentally sustainable systems, planning and legislation will not be sufficient without applied research and innovation.

3.2.5 Conclusions

In conclusion, this study revealed an increase in global interest in RA in recent years, with more projects developing in both the Global North and the Global South, in different climatic areas and independent of city size or demography. However, as in the case of Oceania, national regulations still limit the full development of RA; this highlights the need for policy makers as well as citizens, who often mistrust nontraditional farming systems, to fully comprehend and consider the opportunities that these systems may provide (Sanyé-Mengual et al., 2016). Furthermore, comparing to Global North, the Global South of the world presents a scarce number of cases. This tendency should be taken into account by organizations such as NGOs as well as by local municipalities, in order to use RA as a mean to contrast food insecurity and to create small incomes in depressed areas, as already demonstrated by successful examples described in the paper. The majority of RA projects had a noncommercial farming purpose, especially those established as open-air and soil-based systems. On the other hand, commercial rooftop farms were still underexplored despite the high productive capacity they may provide, especially with the integration of greenhouse technologies and soilless systems. The improvement of RA must build on its proven potential to substantially contribute to providing urban food security and reducing the food miles and environmental impacts associated with current food systems. Accordingly, future research should focus more on how to improve the integration of sustainable practices into RA, such as by developing a functional metabolism between the building and the farmed surface (particularly for water, energy and CO_2 cycles), and how to improve cropping practices by building on existing advances in modern agriculture.

Our findings gave not only a global picture on the current state of the art of RA analyzing the different forms and aspects of its application, but also an objective view on the points that should be implemented in the future to favor effective environmental, economic and social benefits on urban life. Although many countries and governmental institutions are already moving forward a green development of the city context, in other realities some barriers such as old urban plans and codes are still hindering the process. However, the recent events relative to the Covid-19 pandemic bode well for the creation of a new awareness in citizens and policy makers to develop more sustainable and resilient cities. This action should necessarily pass through the rethinking of urban food systems, which suppling food from the inner of urban fabric may help reducing transportation miles as well as impacting on urban micro-climate and citizens' life quality. Therefore, the evolution of RA should be put under a lens in the coming years, being accompanied by further research on sustainability practices and by political decisions in order to achieve a decisive impact on cities regeneration.

3.2.6 Online map

Of the 185 analyzed case studies, 165 reported valid addresses used for the creation of a prototype map. The map was inspired by existing examples (e.g., the Toronto Urban Growers Map) and was created in order to easily locate RA cases worldwide. As this map is a prototype, it should be improved and implemented to become a useful tool for potential users. The map can be found at the following link:

https://www.google.com/maps/d/u/0/viewer?mid=1apMREBaATUTldxyRx7JNg0gasbD-tu1U&ll=2.5756014516108294%2C-125.98531144999993&z=1.

3.3 Chapter 3: Supplemental LED Lighting Improves Fruit Growth and Yield of Tomato Grown under the Sub-Optimal Lighting Condition of a Building Integrated Rooftop Greenhouse (i-RTG)

Elisa Appolloni[1], Ivan Paucek[1], Giuseppina Pennisi[1], Gaia Stringari[2], Xavier Gabarrell Durany[2], Francesco Orsini[1], Giorgio Gianquinto[1]

[1]Alma Mater Studiorum—Università di Bologna, DISTAL—Department of Agricultural and Food Sciences, 48, 40127, Bologna, Italy

[2]Universitat Autònoma de Barcelona, Institut de Ciència i Tecnologia Ambientals (ICTA-UAB), MdM Unit of Excellence (CEX2019-000940-M), Chemical, Biological and Environmental Engineering Department, 08193 Cerdanyola del Vallès, Barcelona, Spain

3.3.1 Abstract

The metabolism of a building can be connected to a rooftop greenhouse, exchanging energy, water and CO_2 flows, therefore reducing emissions and recycling cultivation inputs. However, integrating a rooftop greenhouse onto a building requires the application of stringent safety codes (e.g., fire, seismic codes), to strengthen and secure the structure with safety elements such as thick steel pillars or fireproof covering materials. These elements can shade the vegetation or reduce solar radiation entering the rooftop greenhouse. Nevertheless, application of additional LED light can help to overcome this constraint. The present study evaluated supplemental LED light application in an integrated rooftop greenhouse (i-RTG) at the ICTA-UAB research institute, located in Barcelona (Spain), for tomato cultivation (*Solanum lycopersicum* cv. Siranzo). The experiment explored the effects of three LED lighting treatments and a control cultivated under natural light only (CK). Applied treatments, added to natural sunlight, were: Red and Blue (RB), Red and Blue + Far-Red (FR) for the whole day, and Red and Blue + Far-Red at the end-of-day (EOD), each for 16 h d^{-1} (8 a.m.–12 a.m.) with an intensity of 170 µmol m^{-2} s^{-1}. The results indicate that LED light increased the overall yield by 17% compared with CK plants. In particular, CK tomatoes were 9.3% lighter and 7.2% fewer as compared with tomatoes grown under LED treatments. Fruit ripening was also affected, with an increase of 35% red proximal fruit in LED-treated plants. In conclusion, LED light seems to positively affect the development and growth of tomatoes in building integrated agriculture in the Mediterranean area.

Keywords: Light Emitting Diode, Rooftop Greenhouse, Building-Integrated Agriculture, *Solanum lycopersicum*, Chilling Injury

3.3.2 Introduction

Recent historical events, such as the COVID-19 pandemic and new geo-political arrangements, have demonstrated the need for more resilient food systems capable of ensuring food self-sufficiency, especially in response to sudden changes (Rivera-Ferre et al., 2021). This need for resilience of the food supply chain particularly relates to the urban context, where half of the world’s population is currently living (Knorr et al., 2018). Urban agriculture (UA) (e.g., home gardening, vertical farming, rooftop agriculture) has been identified as a viable solution to promote local production, ensure food security, reduce food waste and create more sustainable food systems (Lal, 2020).

In its most advanced form, UA is applied in or on urban structures as a building-integrated agriculture (BIA) system (Specht et al., 2014). Rooftop farming is an example of BIA, applicable both in unprotected (open-air farms) and protected conditions (rooftop greenhouses) (Appolloni et al., 2021b). The latter case represents a sustainable method of building and cultivation integration, linking the metabolisms of the two systems and symbiotic exchange of CO_2, heat, and water, favoring the optimization of cultivation inputs, energy saving, and emission reduction (Montero et al., 2017). In particular, an integrated rooftop greenhouse (i-RTG) was shown to be able to recycle about 342 kWh m^{-2} yr^{-1} from the main building, which, compared with a traditional fossil-fuel-heated greenhouse, can result in a CO_2 retention of about 114 kg CO_2 (eq) m^{-2} yr^{-1}, corresponding to about 20 EUR m^{-2} yr^{-1} in economic savings (Nadal et al., 2017). However, despite the potential environmental and economic benefits, production in an urban i-RTG may present some limitations due to the shading of surrounding buildings, as well as the bulky structural items, and the loss of transmissivity of fireproof covering materials (e.g., polycarbonate). In fact, given the integration in a city context, the structure must comply with the municipality’s structural and fire safety codes, with consequent constraints affecting the greenhouse light environment (Muñoz-Liesa et al., 2020; Muñoz-Liesa et al., 2021).

Supplementary LED light is receiving wide application in various greenhouse production contexts characterized by reduced solar radiation, such as the cultivation of high-density crops, at high latitudes or during darker seasons (Lu et al., 2012; Zhang et al., 2018; Paponov et al., 2020). In the Mediterranean region, supplementary lighting is still limited in use and applied mainly between October and March (Palmitessa et al., 2021a). However, a more extensive application might be of interest also for the Southern European sector, especially considering the low transmissivity of the chosen plastic roofing materials (60% of PAR reduction) or the need to whitewash greenhouses to protect plants from excessive heat during the summer period (Palmitessa et al., 2021a). These factors, together with increasing competition from Nordic countries, make it necessary for a technological upgrade in the Mediterranean greenhouse sector (Pardossi et al., 2004). Although research has already begun to investigate the effects of supplementary LED light, even in Southern Europe (Paucek et al., 2020; Palmitessa et al., 2020a; Palmitessa et al., 2020b), nothing has yet been demonstrated regarding its application in a low-solar-irradiance BIA context, whether in warm or cold climates. The present research aims to identify the potential benefits and limitations of supplementary LED lighting in the context of agriculture applied to buildings, with a specific focus on the Mediterranean.

3.3.3 Materials and Methods

3.3.3.1 Plants Growing Conditions

The experiment was performed in the i-RTG at the Institute of Environmental Science and Technology (ICTA-UAB) of the Universitat Autònoma de Barcelona (Catalunya, Spain) (41°49′78′′ N, 2°10′89′′ E) (**Figure 17a**). The i-RTG structure consisted of reinforced steel pillars and polycarbonate cladding to satisfy the Spanish Technical Code of Edification and fire safety laws (Muñoz-Liesa et al., 2021).

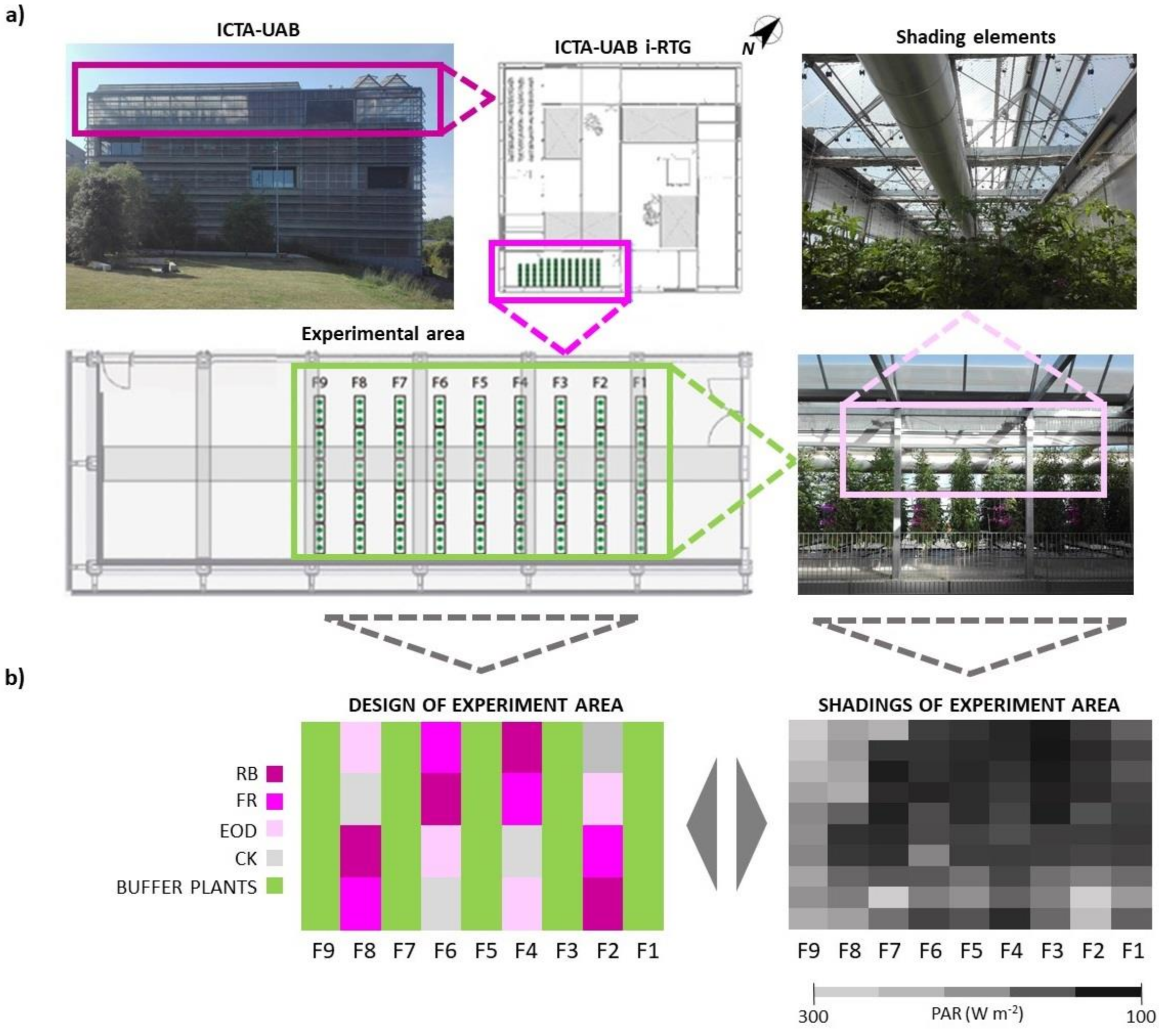


Figure 17. Location, experimental area, and shading elements of the i-RTG at ICTA-UAB (**a**). Experimental design and heatmap of internal shadings of experiment area (**b**).

Tomato plants (*Solanum lycopersicum* L. cv. 'Siranzo', Rijk Zwaan) were cultivated in the south-east oriented corner of the building using a high-wire hydroponic system. Perlite bags (40 L) were used to support plants. Plant distance within rows was 30 cm, while the distance between rows was 80 cm, for a planting density of 3.1 plants m^{-2}. In total, 9 rows (5 m in length each) of plants were cultivated, out of which 5 rows were used as buffers to avoid light treatment pollution among blocks (**Figure 17b**). The plants were sown in peat in late January and transplanted into perlite bags in mid-March. The experiment was terminated at the end of July.

Plants were fertigated with a closed-loop drip irrigation system using the rainwater collected in an underground tank and transported to the top floor by a pump. Irrigation shifts and fertilizer amounts were adjusted during the experiment according to the phenological stage and climatic conditions, maintaining an average pH of 7 and an EC of 1.7 dS m^{-1}. Nutrient solution (**Table 8**), drainage, and rainwater were checked daily to maintain stable nutrition, pH and EC. Temperature (25 ± 4°C), relative humidity (61 ± 14%), and outdoor (294 ± 344 W m^{-2}) and indoor (110 ± 136 W m^{-2}) radiation were constantly monitored using computer-monitored sensors. Passive ventilation was automatically adjusted according to environmental conditions by opening top and lateral windows. The residual heat coming from the lower floors of the building was used to warm-up the greenhouse during cool days.

Table 8. Composition of nutrient solutions during spring and summer period.

	Spring	*Summer*
Composition	kg L^{-1}	kg L^{-1}
KH_2PO_4	0.017	0.014
KNO_3	0.015	0.005
K_2SO_4	0.035	0.035
$Ca(NO_3)_2$	0.049	0.049
$CaCl_2$	0.014	0.014
$Mg(NO_3)_2$	0.011	0.015
Hortrilon	0.001	0.001
Sequestrene	0.001	0.001

3.3.3.2 Light Treatment and Experimental Design

Each light treatment was provided by a couple of LED inter-lighting lamps (Philips GreenPower LED, Philips, Amsterdam, The Netherlands) located at 50 cm and 80 cm of height, and at 30 cm of distance from the stem. A control using natural light only (CK), plus three different supplemental LED lighting regimes, were evaluated. Lighting treatments consisted of:

1. Red (660 nm) and Blue (465 nm) light with R:B ratio of 3, a total photosynthetic photon flux density (PPFD) of 170 µmol m^{-2} s^{-1} (85 µmol m^{-2} s^{-1} per each lamp, measured at 30 cm from the plant) and a photoperiod of 16 h d^{-1} (8 a.m.–12 a.m.) (namely RB);
2. RB treatment with an addition of 40 µmol m^{-2} s^{-1} of Far-Red light (730 nm) during the whole photoperiod (namely FR);
3. RB treatment with an addition of 40 µmol m^{-2} s^{-1} of Far-Red, added only for 30 min right after the end of photoperiod (end-of-day) (namely EOD).

Lighting treatments started in mid-April until the end of the trial. A Latin square design was used to reduce systematic error determined by the shading of air conducts and structural elements on plants (**Figure 17b**). The experiment was divided into 4 replicates (n=4) containing 3 plants per treatment (12 plants per treatment). Lines of buffer plants were used to screen the radiation coming from parallel rows, and 1 or 2 buffer plants were used between two adjacent treatments to avoid light interactions on the row (**Figure 17b**).

3.3.3.3 Plant Vegetative, Physiological and Biochemical Measurements

Stem and collar diameter were measured every two weeks from the beginning of lighting treatment until the end of May, at 1 cm under the fruit truss and 1 cm from the perlite bag, respectively. Internodes length was measured weekly as the distance between two consecutive fruit trusses. Apical growth and the number of clusters were measured every week until plant topping occurred in the last week of June. Final fresh weight of the entire plant biomass (e.g., leaves and stems) was measured with a digital scale at the end of the experiment.

Leaf area was evaluated on the last week of May using Easy Leaf Area software (Department of Plant Sciences, University of California, Davis, CA, USA) (Easlon et al., 2014) on the first leaf above the third fruit truss of each plant. The same leaves were measured as fresh and after drying at 60°C per 4 days. Weight measurements were used to evaluate leaf dry matter content (LDMC), as the ratio between leaf dry mass and leaf fresh mass (mg g^{-1}), and specific leaf area (SLA), as the ratio between leaf area and leaf dry mass (m^2 kg^{-1}) (Granier et al., 2001).

Chlorophyll content of leaves was evaluated in the first week of June, considering three points of the first leaf right under the third fruit truss of each plant. A Chlorophyll Content Meter CCM-200 (Opti-Sciences, Hudson, NY, USA) was used to non-destructively estimate the content based on the ratio of light transmittance at 653 nm and at 931 nm (Denis et al., 2021).

Analysis of micro- and macro-elements (B, Mn, Fe, Cu, Zn, Na, Mg, K, P, S, and Ca) of plant biomass was carried out through a digestion process with HNO_3 and analyzed using ICP-OES optical spectrometry (Optima 4300DV, Perkin-Elmer, Waltham, MA, USA) as reported in Arcas-Pilz et al. (2021). The analysis was conducted on leaf samples collected weekly, and plant stems collected at the end of the experiment. Leaves and stems were analyzed separately.

3.3.3.4 Fruit Development and Yield

Fruit development was monitored on the proximal fruit of the first produced cluster, representative of spring development (Follow-up 1), and the fourth cluster, representative of summer development (Follow-up 2), by measuring the equatorial and polar diameter. Evaluations were taken two times per week during the first three weeks and once a week during the last three weeks, starting from 12 days after anthesis until stabilization of fruit growth (around the turning phase). A digital Vernier caliper was used to measure the polar and equatorial dimensions (cm), estimating the volume of the fruit as the volume of an ellipsoid of rotation $V=(4/3)\ \pi ab^2$, where *a* is one half of the polar radius and *b* is one half of the equatorial radius (Li et al., 2015). Ripening was evaluated on the same fruit two weeks before harvesting using a DA-Meter (SINTELEIA, Bologna, Italy), which non-destructively evaluated the chlorophyll degradation and correlated it with a ripening index.

From the beginning of June until the end of the trial, fruits were harvested once per week (in total 6 clusters per plant). Fresh weight of the total clusters of each treatment was measured with a digital scale. The number of fruits per cluster was counted for each plant. At harvesting, fruits were divided, counted, and weighed as consumable and non-consumable, in which consumable was considered a mature red or dark orange tomato, and not consumable was considered a fruit backward in growth and ripening, mostly green and with small dimensions (<3 cm). This definition was not established on the basis of commercial parameters but rather on the assessment of the edibility of tomatoes to be used for local and direct consumption by the office workers. The average fresh weight of an individual fruit per treatment was estimated by dividing the total fresh weight by the total number of fruits. The total fresh mass of fruit produced by the plant was estimated by multiplying the number of fruits of each plant by the average fresh weight of an individual fruit for each treatment.

3.3.3.5 Fruit Quality and Biochemical Analysis

Fruit Qualitative Evaluation

Fruit qualitative evaluations were performed two times on mature tomatoes (spring development, Follow-up 1, and summer development, Follow-up 2), and one time on immature tomatoes at the turning stage (summer development, Follow-up 2). Mature tomatoes were selected considering a DA-Meter range index between 1.30 and 1.50, whereas immature tomatoes were selected considering a range index between 0.20 and 0.40.

Fruit hardness was evaluated using a fruit hardness tester (Turoni, Forlì, Italy) on four opposite sides of the equatorial diameter of the fruit. The instrument non-destructively measured the elasticity of fruit exocarp, expressing it as an index ranging from 0 to 100. Soluble solid content and acidity were measured on fruit juice using a pocket Brix and acidity meter (PAL-BX|ACID3, Atago, Tokyo, Japan). Fruit dry matter content (FDMC) was measured as the ratio between fruit fresh weight and dried fruit.

Lycopene and β-Carotene Content

Lycopene and β-carotene content were evaluated on tomato samples frozen at −20°C, using the methodology described by Anthon and Barrett (Anthon and Barrett, 2006), with slight modifications. Briefly, an extraction solution was prepared by mixing hexane, acetone, and ethanol in a v:v proportion of 2:1:1 and 0.5 g L^{-1} of butylated hydroxytoluene (BHT). Thereafter, 0.5 g of frozen sample including exocarp and mesocarp, trying to avoid the green parts (e.g., petiole), were pestled and mixed with 10 mL of extraction solution. The material was left in darkness for 30 min and then centrifuged at 5000 rpm for 5 min. Finally, 1 mL of supernatant was read at 503 nm for lycopene and 444 nm for β-carotene with a spectrophotometer (8453 UV-Visible Spectrophotometer, HP, Palo Alto, CA, USA).

The lycopene content was calculated using the following formula:

$$\text{lycopene}\left(\frac{\text{mg}}{\text{kg}}\right)=\left(\frac{\text{X}}{\text{Y}}\right)\times\text{A503}\times 3.12$$

where X is the amount of hexane (mL), Y the weight of the fruit tissue (g), A503 is the absorbance at 503 nm, and 3.12 is the extinction coefficient. β-Carotene was calculated with the following equation:

$$\beta\text{- carotene}=(9.38\ \times\text{A444}-6.70\ \times\text{A503})\times 0.55\ \times 537\ \times\frac{\text{V}}{\text{W}}$$

where A444 is the absorbance at 444 nm, A503 is the absorbance at 503 nm, 0.55 is the ratio of the final hexane layer volume to the volume of mixed solvents added for hexane:acetone:ethanol (2:1:1), V is the volume of mixed solvents added, W is the fresh weight of the sample, and 537 (g mole^{-1}) is the molecular weight of β-carotene.

Total Polyphenols and Total Antioxidant Capacity

Total polyphenols and total antioxidant capacity were evaluated on tomato samples frozen at −20°C. The extraction was performed by placing 4 g of samples in tubes and adding 8 mL of extraction mixture (60% methanol, 30% H_2O, 10% acetone). The process was carried out by centrifugation at 5000 rpm for 10 min at 4°C. Supernatant was collected and used for antioxidant and total phenol analysis.

Total antioxidant capacity was analyzed using the FRAP (Ferric Reducing Antioxidant Power) method, developed following the method of Benzie and Strain (Benzie and Strain, 1999), with slight modifications. Briefly, a reaction mixture containing acetate buffer (pH 3.6), 300 mM of 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) solution (in 40 mM HCl) and 20 mM $FeCl_3$ was prepared in a v:v:v proportion of 10:1:1 and incubated at 37°C for 2 h in darkness. Then, 1.2 mL of reaction mixture was added to 20 µL of supernatant and incubated for 1 h at room temperature in darkness. Calibration standards were prepared by dissolving 28 mg of $FeSO_4$ in 10 mL of H_2O (10 mM) and subsequently diluting 1 mL of the solution with 1 mL of H_2O (5 mM). Six Eppendorf tubes containing 40 µL of H_2O were prepared and 20 µL of 5 mM solution were added to the first Eppendorf (2.5 mM). The operation was repeated moving 20 µL of solution, from one tube to another 6 times, in order to halve the concentration at each dilution. A blank with 40 µL of H_2O was also prepared. Samples and standards were read at 593 nm with a spectrophotometer (8453 UV-Visible Spectrophotometer, HP, Palo Alto, CA, USA).

Total polyphenols were determined using the methodology described by Waterhouse (Waterhouse, 2002), with slight modifications. Briefly, 50 µL of sample extract were added to 800 µL of extraction mixture (H_2O and Folin–Ciocalteu reagent in a v:v proportion of 15:1). Calibration standards were prepared by dissolving 80 mg of gallic acid in 10 mL of H_2O (8 mg mL^{-1}) and subsequently diluting 0.5 mL of the solution with 4.5 mL of H_2O (800 µg mL^{-1}). Six Eppendorf tubes containing 50 µL of H_2O were prepared and 50 µL of 800 µg mL^{-1} solution were added to the first (400 µg mL^{-1}). The operation was repeated moving 50 µL of solution from one tube to another for 6 times, in order to halve the concentration at each dilution. A blank with 50 µL of H_2O was also prepared. After an incubation of 5 min, samples and standards were added with 150 µL of 20% Na_2CO_3, incubated for 1 h at room temperature and then read at 765 nm with a spectrophotometer (8453 UV-Visible Spectrophotometer, HP, Palo Alto, CA, USA).

3.3.3.6 Chilling Injury Analysis

In mid-June, both mature (DA-Meter Index 1.30–1.50) and immature tomatoes (DA-Meter Index 0.20–0.40) were harvested and stored for one week at 4°C. After cold storage, fruits were moved into a dark room at 20°C, 60% RH and 600–660 ppm of CO_2 for 2 weeks. Measurements took place right after 4°C chilling (T1) and 10 days after 20°C storage (T2).

Non-destructive analyses, including fresh weight loss, hardness, and ripening, were performed on the same tomatoes at T1 and T2 as described above. Fruit fresh weight was also evaluated before chilling. Destructive analyses, including soluble solids, lycopene content, β-carotene content, antioxidant activity, and total phenol content, were performed at the end (T2) of chilling injury evaluation as described above.

Chilling injury index was visually attributed at T2 according to Vega-Garcìa et al. (2010) and Affandi et al. (2020). In particular, the criteria to evaluate the symptoms of chilling damage consisted of: uneven ripening and color development (U), pitting (P), and decay (D). A five-point scale was used to attribute the severity of the symptoms based on the percentage of affected fruit surface: 0 = no injury, 1 ≤ 10%, 2 = 11% to 25%, 3 = 26% to 40%, and 4 ≥ 40%.

3.3.3.7 Energy Cost Assessment

The energy cost assessment was calculated based on the actual consumption of a lamp with standard RB treatment (0.044 kW) applied for 16 h per day (0.704 kWh d^{-1}). Costs were estimated per plant per day, assuming that treatments were carried out with double lamps and that each pair of lamps covered about four plants. The price of electricity was acquired from EUROSTAT (2021) dataset considering household electricity prices for Italy (0.176 € kWh^{-1}) and Spain (0.188 € kWh^{-1}) in 2021, the two main producers of tomato in the Mediterranean area.

3.3.3.8 Statistical Analysis

Statistical analysis was performed by applying one-way ANOVA and Tukey’s test to compare means. Data were analyzed by using SPSS software. The Marascuilo procedure was used to compare multiple proportions in case of maturation degree and chilling injury index evaluation. Statistics considered a 5% significance level ($p \leq 0.05$).

3.3.4 Results

The influence of LED light on plants vegetative parameters was not significant. In particular, stem and collar diameter measurements showed no significant differences between light treatments and unlighted control at each time point (data not shown). Concurrently, an absence of differences was observed in the average internode length, although the third internode of CK plants showed a significant increase in length compared with RB treatment, featuring mean lengths of 31 ± 2 and 27 ± 4 cm, respectively. This difference disappeared in the following internodes. However, the average elongation of the plant apex showed a significantly greater length in the unilluminated control than in the RB treatment, with mean lengths of 29 ± 3 and 25 ± 3 cm, respectively. The final fresh weight of the entire plant, leaves and stems, did not show any difference (data not shown). In the same way, leaf area, LDMC, SLA, and chlorophyll content did not present statistically significant changes among treatments. Lighting regimes did not affect leaves' micro- and macro-elements content (**Table 9**). At the same time, stems presented significantly different accumulations of Fe, Cu, Mg, K, and P elements depending on light treatment. In particular, Cu and P were shown to have a greater accumulation in CK plants than in LED-treated plants, as reported in **Table 9**.

Table 9. Mineral element contents (mg L^{-1}) in leaves and stems of tomato plants grown under different lighting treatments (different letters indicate significant differences at $p \leq 0.05$, SE = Standard Error).

	B	Mn	Fe	Cu	Zn	Na	Mg	K	P	S	Ca
	Leaves										
CK	1.0^{a}	0.5^{a}	3.0^{a}	0.2^{a}	0.5^{a}	51.2^{a}	62.3^{a}	465.5^{a}	49.6^{a}	213.7^{a}	595.4^{a}
RB	0.9^{a}	0.4^{a}	3.7^{a}	0.3^{a}	0.5^{a}	42.2^{a}	64.3^{a}	391.1^{a}	38.5^{a}	195.5^{a}	573.8^{a}
FR	0.9^{a}	0.4^{a}	2.7^{a}	0.2^{a}	0.5^{a}	47.1^{a}	66.5^{a}	413.5^{a}	41.9^{a}	189.2^{a}	559.5^{a}
EOD	1.0^{a}	0.5^{a}	3.6^{a}	0.2^{a}	0.4^{a}	51.9^{a}	66.3^{a}	426.1^{a}	42.6^{a}	199.5^{a}	590.9^{a}
SE	0.1	0.0	0.6	0.1	0.0	6.1	4.6	32.1	4.9	15.3	40.1
	Stem										
CK	0.5^{a}	0.1^{a}	0.4^{b}	0.2^{a}	0.5^{a}	53.4^{a}	36.2^{b}	498.8^{ab}	62.3^{a}	30.2^{a}	139.7^{a}
RB	0.5^{a}	0.2^{a}	0.7^{a}	0.1^{b}	0.5^{a}	46.3^{a}	43.5^{a}	536.1^{a}	52.8^{ab}	34.4^{a}	154.2^{a}
FR	0.5^{a}	0.1^{a}	0.4^{b}	0.1^{b}	0.4^{a}	55.3^{a}	37.7^{ab}	421.8^{b}	45.7^{b}	28.6^{a}	117.6^{a}
EOD	0.5^{a}	0.1^{a}	0.4^{b}	0.1^{b}	0.3^{a}	48.3^{a}	32.0^{b}	442.6^{ab}	47.0^{b}	26.9^{a}	112.9^{a}
SE	0	0.1	0.1	0.0	0.1	9.0	2.5	40.3	5.3	3.5	19.9

The increase in volume of proximal fruits showed different trends depending on the season. In particular, fruits of Follow-up 1, representative of the spring season, were not affected by the lighting regimes (data not shown). In contrast, fruits of Follow-up 2, representative of the summer period, showed clear differences in growth, particularly comparing CK and RB treatments (**Figure 18**). Evaluation of ripening degree showed the same seasonal difference, demonstrating no difference for representative spring season fruit. In contrast, summer fruit seemed to present a faster ripening when exposed to LED light treatments, showing 35% more red fruit compared with CK plants (**Figure 19**).

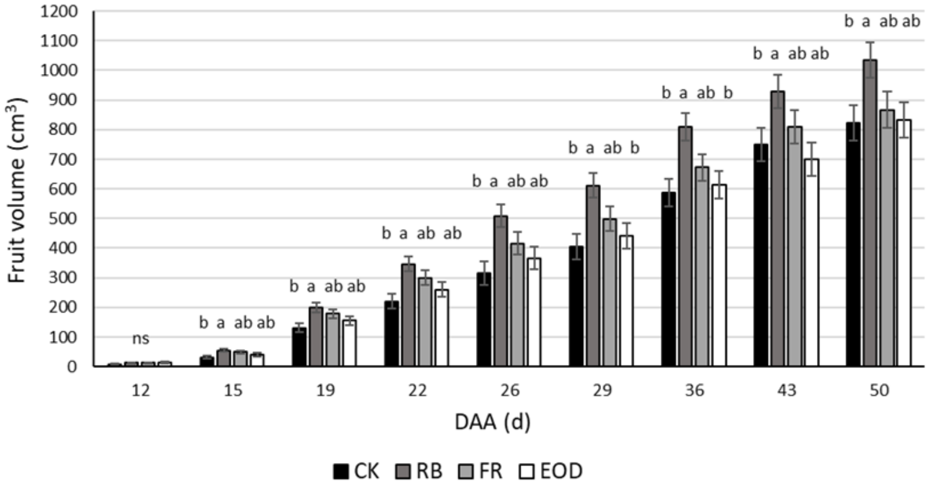
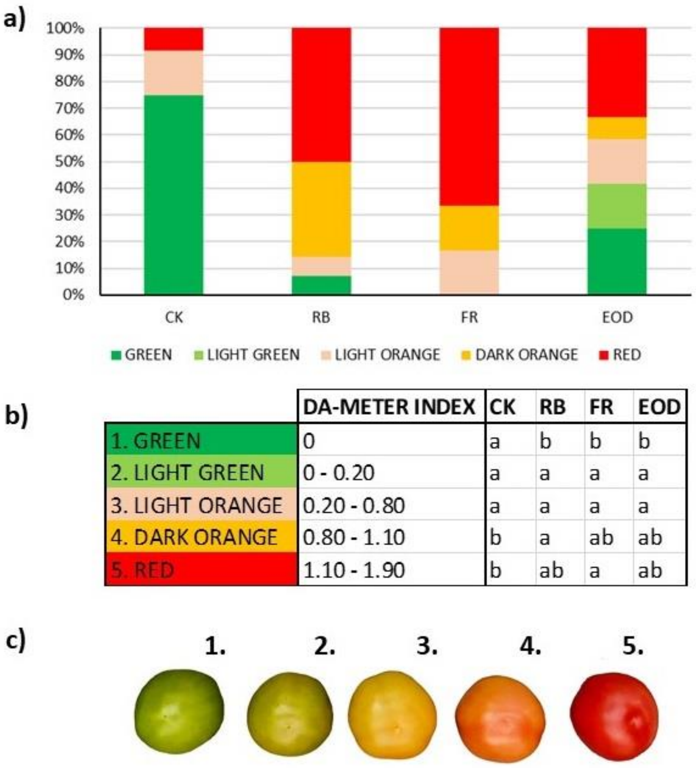


Figure 18. Volume development of representative fruit of summer period (Follow-up 2) depending on days after anthesis (DAA) ($p \leq 0.05$).

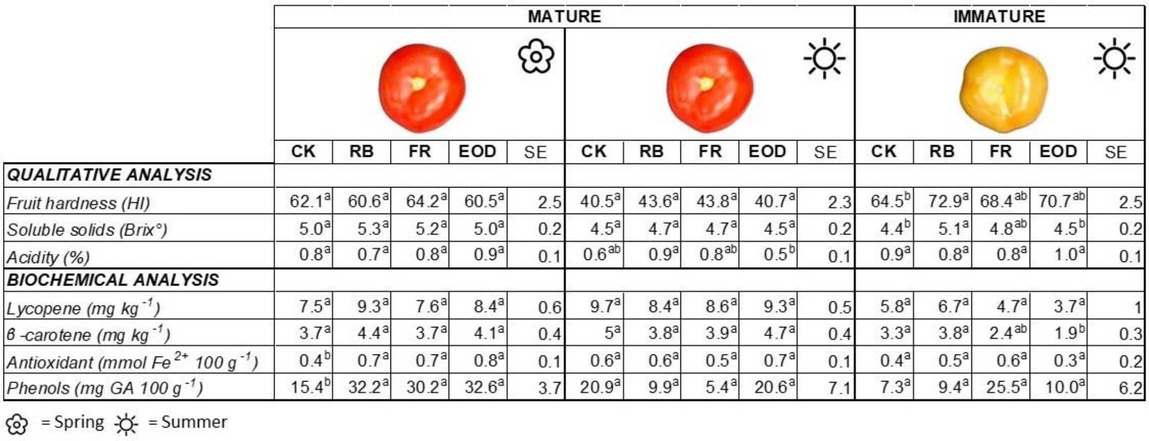


	DA-METER INDEX	CK	RB	FR	EOD
1. GREEN	0	a	b	b	b
2. LIGHT GREEN	0 - 0.20	a	a	a	a
3. LIGHT ORANGE	0.20 - 0.80	a	a	a	a
4. DARK ORANGE	0.80 - 1.10	b	a	ab	ab
5. RED	1.10 - 1.90	b	ab	a	ab

Figure 19. Comparison of multiple proportions **(a)** and respective significance ($p \leq 0.05$) **(b)** for five tomato maturation classes **(c)** depending on lighting treatment applied to fruits of Follow-up 2.

Control tomatoes were approximately 9.3% lighter than tomatoes grown under light treatments (approximately 93.8 g for CK and 103.5 g in LED-treated plants). Fruit number was also lower (−7.2%) in control plants than in LED-treated plants (38.4 fruits plant^{-1} in CK and 41.4 fruits plant^{-1} in LED-treated plants). The statistical evaluation shows a significantly higher yield of LED-treated plants (RB, FR, and EOD) as compared with those grown with natural light only (CK). In particular, plants grown without supplemental LED light showed around 17% lower yield as compared with those grown with LED light (3.6 kg plant^{-1} in CK and 4.4 in LED-treated plants). Regarding yields for consumable and non-consumable tomatoes of each treatment, plants treated with LED lights produced a lower number of consumable red tomatoes (−9.3%) than control plants, although these fruits were larger in size (+10.5%). On the contrary, non-consumable green tomatoes of LED treatments were higher in number (+8.8%) than those in control, still presenting a larger average size (+13.7%).

Qualitative and biochemical parameters showed different trends depending on the period in which the fruits developed (e.g., spring or summer) and the ripening stage at harvest (e.g., mature or immature) (**Figure 20**). In particular, observing representative fruits from the spring period, no significant differences were observed concerning the evaluation of qualitative parameters. However, a significantly lower content of antioxidants and phenols was observed in control fruits' phenol content and antioxidant capacity compared with those grown with LED light treatments (**Figure 20**). In the case of summer fruits harvested at the mature stage, qualitative analyses showed lower acidity in fruits from the EOD treatment than the RB treatment (a reduction by 44.4% in EOD compared with RB), but no difference at the biochemical level. Finally, the evaluation of immature fruits, carried out only for the summer period, showed significantly higher hardness in fruits obtained from the RB treatment as compared with those produced by CK plants (11.5% less in CK compared with RB), and significantly higher soluble solid content in the RB than CK and EOD (13.7% less in CK and EOD compared with RB). For the biochemical analysis, the immature fruits from the summer period had a statistically significantly lower β-carotene content than RB and CK treatments (**Figure 20**). FDMC of selected fruits did not show any significant difference among treatments (data not shown).



	MATURE										IMMATURE				
	✿					☼					☼				
	CK	**RB**	**FR**	**EOD**	SE	**CK**	**RB**	**FR**	**EOD**	SE	**CK**	**RB**	**FR**	**EOD**	SE
QUALITATIVE ANALYSIS															
Fruit hardness (HI)	62.1^{a}	60.6^{a}	64.2^{a}	60.5^{a}	2.5	40.5^{a}	43.6^{a}	43.8^{a}	40.7^{a}	2.3	64.5^{b}	72.9^{a}	68.4^{ab}	70.7^{ab}	2.5
Soluble solids (Brix°)	5.0^{a}	5.3^{a}	5.2^{a}	5.0^{a}	0.2	4.5^{a}	4.7^{a}	4.7^{a}	4.5^{a}	0.2	4.4^{b}	5.1^{a}	4.8^{ab}	4.5^{b}	0.2
Acidity (%)	0.8^{a}	0.7^{a}	0.8^{a}	0.9^{a}	0.1	0.6^{ab}	0.9^{a}	0.8^{ab}	0.5^{b}	0.1	0.9^{a}	0.8^{a}	0.8^{a}	1.0^{a}	0.1
BIOCHEMICAL ANALYSIS															
Lycopene (mg kg $^{-1}$)	7.5^{a}	9.3^{a}	7.6^{a}	8.4^{a}	0.6	9.7^{a}	8.4^{a}	8.6^{a}	9.3^{a}	0.5	5.8^{a}	6.7^{a}	4.7^{a}	3.7^{a}	1
β -carotene (mg kg $^{-1}$)	3.7^{a}	4.4^{a}	3.7^{a}	4.1^{a}	0.4	5^{a}	3.8^{a}	3.9^{a}	4.7^{a}	0.4	3.3^{a}	3.8^{a}	2.4^{ab}	1.9^{b}	0.3
Antioxidant (mmol Fe $^{2+}$ 100 g $^{-1}$)	0.4^{b}	0.7^{a}	0.7^{a}	0.8^{a}	0.1	0.6^{a}	0.6^{a}	0.5^{a}	0.7^{a}	0.1	0.4^{a}	0.5^{a}	0.6^{a}	0.3^{a}	0.2
Phenols (mg GA 100 g $^{-1}$)	15.4^{b}	32.2^{a}	30.2^{a}	32.6^{a}	3.7	20.9^{a}	9.9^{a}	5.4^{a}	20.6^{a}	7.1	7.3^{a}	9.4^{a}	25.5^{a}	10.0^{a}	6.2

✿ = Spring ☼ = Summer

Figure 20. Qualitative and biochemical evaluation of mature and immature tomatoes representative of spring (Follow-up 1) and summer (Follow-up 2) periods (different letters indicate significant differences at $p \leq 0.05$, SE = Standard Error).

Cold storage showed different results on tomatoes depending on the ripeness degree. In particular, immature tomatoes showed no significant differences over time, except for polyphenol content. Indeed, polyphenols were found to be statistically lower in EOD and FR treatments compared with RB at T2, whereas no differences were observed among LED treatments and control (31.8 ± 8 mg GA 100 g^{-1} in CK, 39.2 ± 6 in RB, 26.1 ± 4 in FR and 19.9 ± 7 in EOD). Immature fruits also showed more irregular ripening with the application of additional LED treatments, whereas no uneven ripening was observed in CK fruit (**Figure 21a**). No significant statistical changes were observed in the case of pitting or decay evaluation for immature tomatoes (data not shown). On the other hand, ripe fruits showed significantly higher weight loss in CK compared with FR and EOD treatments immediately after 4°C chilling (T1) (1.6 ± 0.1% in CK, 1.1 ± 0.4 in RB, 0.7 ± 0.3 in FR, and 0.6 ± 0.4 in EOD). However, differences among treatments disappeared after 10 days (T2). Differences in mature tomatoes were also observed in the case of β-carotene content, where RB treatment showed a significantly higher concentration than EOD treatment at T2 (4.9 ± 0.7 mg kg^{-1} in CK, 5.9 ± 0.9 in RB, 4.6 ± 0.7 in FR, and 4.1 ± 0.9 in EOD). Regarding chilling injury assessment, ripe tomatoes treated with RB light showed a significantly higher number of fruits in the intermediate class of decay compared with the other treatments 10 days after chilling (T2) (**Figure 21b**). However, although not significant, CK presented 80% more fruits in the highest decay class compared with fruits treated with RB light. No significant changes were observed in the case of pitting and uneven ripening evaluation for ripened tomatoes (data not shown).

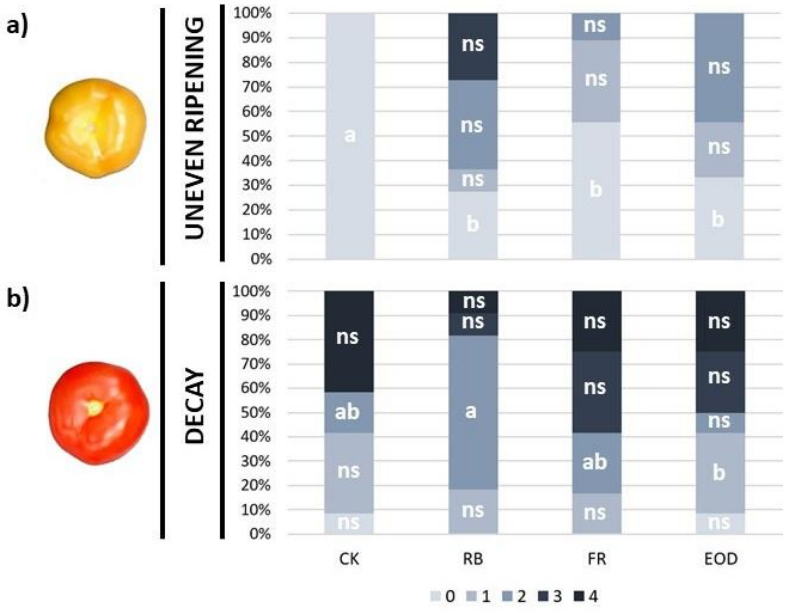


Figure 21. Comparison of multiple proportions and respective significance ($p \leq 0.05$) of five chilling injury classes (0–4) depending on lighting treatment. Reported chilling injury refers to uneven ripening of immature fruit **(a)** and decay of mature fruit **(b)**.

Evaluation of energy consumption per plant showed an average value of about 0.022 kW, which multiplied by 16 h of treatment results in a daily consumption of about 0.352 kWh per plant. Considering this consumption and the household electricity costs reported above (EUROSTAT, 2021), the daily cost to obtain a yield increase of 17% during the spring–summer period is about 0.06 EUR plant^{-1} d^{-1} for Italy and 0.07 EUR plant^{-1} d^{-1} for Spain, resulting in an increased cost by 1.27 EUR kg^{-1} in Italy and 1.35 EUR kg^{-1} in Spain.

3.3.5 Discussions

The amount of light radiation intercepted by plants depends on both the leaf area index (LAI, total leaf area per unit ground area) and a light extinction coefficient (k) influenced by morphological factors (Hirose, 2005). The capacity to modify plant architecture and consequently increase light interception is fundamental for improving photosynthetic rate. Specific wavelengths are known to influence and modify some vegetative and morphological traits of tomato plants. In particular, Far-Red radiation has been shown to have effects on the elongation of tomato internodes, leaf morphology, and inclination (Zhang et al., 2018; Kurepin et al., 2010; Hao et al., 2016) being related to the so-called shade avoidance syndrome (Franklin and Whitelam, 2007). This phenomenon is determined by a low R:FR perception by plant phytochromes, triggering different responses that also involve leaf development, apical dominance, internode extension, chloroplast development, and assimilate partitioning, among others (Smith and Whitelam, 1997). In natural conditions, without the artificial addition of Far-Red light, this phenomenon can be triggered by the Far-Red reflected by the leaves of the surrounding canopy as a signal of competition (Ballaré et al., 1990). Furthermore, a natural low R:FR ratio can also occur at the end of the day (Holmes and Smith, 1977), when the sun is low and longer wavelengths can travel further in the atmosphere.

In this report, the effects of different LED supplemental lighting spectral conditions, with or without Far-Red, have been evaluated on greenhouse tomato growth. Most vegetative and physiological traits were not affected by lighting regimes, contrary to what was reported by other researches, where some parameters, such as plant height or leaf area, were increased by Far-Red addition as compared with Red and Blue treatment alone (Kalaitzoglou et al., 2019). The average apical elongation was the only parameter showing statistically significant differences, resulting in CK values higher than RB, but not than FR and EOD, as a possible consequence of the elongation effect given by Far-Red presence. On the other hand, the absence of a statistical difference among FR and EOD compared with RB may be attributed to the presence of the Blue wavelength beside Far-Red light, known to have a possible dwarfing effect on plants, therefore lowering LED-treated plants' height (Ahmad et al., 2002). Concerning mineral element evaluation, previous studies suggested that light treatments might impact their accumulation in some plant organs (Amoozgar et al., 2017; Palmitessa et al., 2021b). In particular, Samuolienė et al. (2021) observed a higher accumulation of elements in tomato leaves grown with supplemental green LED light. In the present research, however, no differences were observed in leaves, although the different lighting treatments seem to have played different effects on the accumulation of specific elements in plant stems (**Table 9**).

Supplemental LED light can increase the photosynthetic rate of tomato plants and consequently influence fruit size and weight (Jiang et al., 2017). However, the effect of light on fruit growth can be affected by its position on the cluster, given the greater sink strength that a proximal fruit can have compared with a distal fruit. The sink strength of proximal fruit is related to higher cell division due to competitive assimilating processes triggered in the first phase of fruit development after pollination (Bertin et al., 2002). In the following stages, the fruit accumulates most of its dry matter and undergoes a process of cell enlargement until it reaches the final stage of ripening, where it stops accumulating carbohydrates (Ho et al., 1986). In high-wire systems, where tomato plants are periodically lowered, fruits in the cell division stage are at the top of the plant, away from the LED lamps, whereas during the cell enlargement stage, fruits are at the bottom of the plant near the lamps. It might be possible to think that the lamps can only affect the second development stage, being in direct contact with the fruits. However, when comparing the volume growth over time of representative tomato fruits from spring (Follow-up 1) and summer (Follow-up 2) phases, different conclusions may be drawn. Whereas earlier in the season no significant differences were observed between treatments, later, RB treatment showed a significantly greater dimension of fruits than CK, already after the second measurement (T2) (**Figure 18**). Whereas the light treatment for spring fruits was started after anthesis, on already formed fruit, in summer fruits, the treatment was already in progress since the early flowering. From these observations, it can be deduced that, despite being distant from the lamps, summer fruits have been affected by LED light, accumulating a major quantity of assimilates since the cell division stage. A similar response was also reported by Paponov et al. (2020), although differences were mainly observed in fruits at the intermediate position in the cluster.

The presence of far-red might influence the enhancement of tomatoes fresh weight [Hao et al., 2016; Fanwoua et al., 2019). However, as formerly observed in other studies performed in the Mediterranean area (Palmitessa et al., 2020b), the presence of additional far-red did not seem to have specifically affected plant yield. However, a general increase in average fruit weight and fruit number was observed in all LED treatments compared with the control. Total yield had a percentage increase (17%) very similar to those observed in other studies comparing the use of supplemental LED light with natural light alone (Pucek et al., 2020; Palmitessa et al., 2020a; Jiang et al., 2017; Pepin et al., 2013; Hao et al., 2017). By dividing the number of consumable fruits from non-consumable fruits, it was observed that plants under LED treatment produced fewer but larger Red tomatoes, and more and bigger green tomatoes than the control. This observation seems to confirm the previous results by Paponov et al. (2020) on the ability of LED light to increase the number of cells and thus the sink strength in the early developmental stage of fruit at a different height on the truss. During the ripening stage, although fewer in number than the control, proximal fruits of LED-treated plants were found to have an earlier ripening (**Figure 19**) as already observed by some authors (Paucek et al., 2020; Zhang et al., 2020). The higher ripening rate in proximal fruits of LED-treated plants could be attributed to a greater competition and consequent accumulation of some biochemical compounds such as phytoene or melatonin, responsible for ripening processes (Zhang et al., 2020; Li et al., 2021a).

Additional LED light is known to positively affect several quality attributes of tomatoes, although some inconsistencies among published research have been shown, probably determined by different environmental conditions and genotypes (Palmitessa et al., 2021a; Appolloni et al., 2021a). On ripe fruits, a significant increase in antioxidant capacity and phenol content in the LED-treated fruits was observed in spring, but no significant differences between lighting treatments was detected in the summer (**Figure 20**). The effect of LED light on qualitative attributes may also change according to the fruit ripening stage, as observed in immature tomatoes in relation to sugar content and fruit hardness. Accordingly, Fanwoua et al. (2019) already observed that the effects of LED light on pericarp sugar concentrations could change based on fruit age, as a consequence of different fruit water content that would affect the sugar dilution.

To the best of our knowledge, limited research has investigated the effects of supplemental LED light applied at the cultivation stage on chilling injury and the post-harvest quality of tomatoes. In particular, Affandi et al. (2020) observed that high levels of supplemental Far-Red light might improve cold tolerance in both mature Green and Red tomatoes, reducing pitting, decay, and weight loss. In our research, a significant effect on cold tolerance was not observed. However, Far-Red light, applied both throughout the day and at the end-of-the-day, would seem to confirm an ability to delay weight loss, especially during the chilling phase (T1). In contrast to a former report by Affandi et al. (2020), mature Green tomatoes treated with Far-Red light did not seem to have a faster turning to Red compared with RB treatment, whereas the best performance was observed in the CK case.

Based on the results of our research, the application of additional LED light seems not economically feasible. In fact, considering the productive capacity of our experiment, the energy cost per kg is 1.27 EUR kg^{-1} in Italy and 1.35 EUR kg^{-1} in Spain, whereas selling prices reported by EUROSTAT (2021) in 2021 are 1.15 EUR kg^{-1} and 0.74 EUR kg^{-1} for Italy and Spain, respectively. Other authors have already observed scarce economic feasibility, estimating energy consumption of about 28.8 kWh to increase tomato yield by 1 kg (Yan et al., 2018). However, these observations need to be put into context. In particular, the country's socio-economic condition, geo-political situation, access to renewable energy, as well as the cultivation latitude, and greenhouse light accessibility and transmissivity, may all influence the feasibility of supplementary LED lighting application to increase tomato yield. Besides that, greenhouse design, management of cultivation period, and lighting strategy are other important aspects to adapt to maximize the economic benefit. For instance, it has been observed that in a Northern European context, a year-round production with high technology investment (e.g., supplemental lighting, heat pump) can provide the most elevated economic return (Naaser et al., 2022). This contextual approach could also be applied in the Mediterranean area, modifying some parameters according to the different climatic and socio-economic conditions. To give an example, our study looked only at the spring–summer time span, although the application during the cold-season could have resulted in more cost-effective results. Furthermore, our research referred to household on-grid electricity costs, applied to an urban BIA context intended for direct consumption by building users. Integration of alternative energy sources such as solar panels may drastically reduce the electricity cost, leading to a net profit increase both at domestic and commercial levels. Integrating the research with different lighting strategies (e.g., pulsed light, cold-season lighting, photoperiod reduction) should be considered better to estimate the economic benefits for the Mediterranean area.

3.3.6 Conclusions

The application of supplementary LED light for tomato cultivation in a BIA context, subjected to a higher reduction of the solar radiation entering the greenhouse, has been shown to increase yields by 17% regardless of the type of treatment used (with or without Far-Red, during the whole day or at the end-of-the-day). Additional light also showed an ability to increase the dimension and number of tomatoes, and speed up the ripening process, particularly in the case of proximal fruits. From an economic point of view, the application of supplementary light would seem to have low feasibility if applied during the spring–summer period. However, applying different and contextualized lighting strategies could lower energy costs. Therefore, future research should focus on more economically valuable methods of managing supplementary lighting, for example, the use during the winter period, different photoperiods and intensities, or techniques that can provide energy savings, such as pulsed light.

3.4 Chapter 4: Potential application of pre-harvest LED interlighting to improve tomato quality and storability

Elisa Appolloni[1], Giuseppina Pennisi[1], Ivan Paucek[1], Antonio Cellini[1], Andrea Crepaldi[2], Francesco Spinelli[1], Giorgio Gianquinto[1], Xavier Gabarrell[3], Francesco Orsini[1]

[1]Alma Mater Studiorum – Università di Bologna, DISTAL – Department of Agricultural and Food Sciences, Bologna, Italy

[2]Flytech srl, Via dell'Artigianato, 65, 32016 Alpago, Belluno, Italy

[3]Universitat Autònoma de Barcelona, Institut de Ciència i Tecnologia Ambientals (ICTA-UAB), MdM Unit of Excellence CEX2019-000940-M; Chemical, Biological and Environmental Engineering Department, Barcelona, Spain

3.4.1 Abstract

Growing conditions and agronomical input play a key role in determining fruit qualitative and nutraceutical traits at harvest and post-harvest. The hereby presented research investigated the effects of pre-harvest supplemental LED interlighting on post-harvest quality of hydroponically grown tomatoes (*Solanum lycopersicum* "Siranzo"). Three LED treatments were added to natural sunlight applied for 16 h d^{-1} (h 8.00-00.00) and consisted of Red and Blue (RB), Red and Blue + Far-Red (FR), and Red and Blue + Far-Red at the end-of-day for 30 minutes (EOD), with an intensity of 180 µmol s^{-1} m^{-2} for Red and Blue, plus 44 µmol s^{-1} m^{-2} for Far-Red. A control treatment (CK), where plants were grown only with sunlight, was also considered. Fruit at red stage were selected and placed in a storage room at 13°C in darkness. Fruit quality assessment was performed at harvest time and after one week of storage. RB and FR increased fruit firmness compared to CK, opening possible benefits toward reducing fruit losses during post-harvest handling. RB treated fruit also maintained a higher content of lycopene and β-carotene after the first week of storage. The study demonstrates that supplementary LED interlighting during greenhouse tomato cultivation may enhance storability and help conserve fruit nutritional properties during post-harvest.

Keywords: Supplemental lighting, Light Emitting Diode, Post-Harvest, *Solanum lycopersicum*, Food waste.

3.4.2 Introduction

Post-harvest management is essential in maintaining tomato quality, ensuring long-term shelf-life, and reducing food waste from unsold and perishing products. Indeed, post-harvest losses of tomatoes can reach 25-40% of production (Khan and Jan, 2007), with economic consequences relapsing on farmers, the processing industry and traders (Kader et al., 1992). Cold storage, high carbon dioxide atmosphere, calcium chloride application and relative humidity control are some methods applied to reduce tomato's respiratory metabolisms and consequent food losses during post-harvest handling (Toor and Savage, 2006; Isaac et al., 2015). In particular, a storage temperature between 10-15°C is the most applied for post-harvest preservation (Mata et al., 2019). Indeed, this is the ideal temperature to ensure the long-term preservation of the fruit without impairing tomato flavor properties (Maul et al., 2000). Furthermore, storage temperatures lower than 13°C can determine chilling injury, affecting tomato texture, surface pitting and fungal development (Mata et al., 2019).

Pre-harvest conditions and agricultural inputs are also crucial in determining fruit quality at harvest and post-harvest ripening (Isaac et al., 2015). For example, fertilizers may help prevent disease development (e.g., calcium) (Passam et al., 2007) or reduce ripening and color formation (e.g., nitrogen); flowers and fruit pruning help obtain an appropriate source/sink ratio and allow to increase fruit weight and dry matter content (Hanna, 2009); and correct irrigation management can increase sugar concentration in fruit (Mitchell et al., 1991). The maturity stage at which the fruit is harvested is another important pre-harvest factor that can influence post-harvest preservation. In particular, harvesting tomatoes at mature green stage can ensure a longer product shelf-life (Moneruzzaman et al., 2009), thanks to a lower sugar content that makes the fruit less vulnerable to mechanical damage (Toivonen, 2007). However, early harvests were also shown to reduce the nutritional and sensorial properties of the product (Balibrea et al., 2006; Isaac et al., 2015), with negative consequences on consumers' perception and nutritional value. Therefore, harvesting at mature-green stage can be advisable in case of product transportation at long distances while harvesting at red ripen stage is the best option for local consumption (Moneruzzaman et al., 2009; Isaac et al., 2015).

Light is a key factor in determining fruit quality being the main responsible for photosynthetic activity. In recent years, the development of highly efficient and low-cost light-emitting diodes (LEDs) has allowed the adoption of this technology for protected crop production (Paradiso and Proietti, 2021), fostering the study of how light manipulation may affect plant performances and fruit quality, particularly in tomatoes (Appolloni et al., 2021a). Furthermore, LED light has also been applied to harvested tomatoes during storage to enhance berry quality and storage (Cozmuta et al., 2016; Ngcobo et al., 2021). Chlorophylls absorption peaks correspond to the Blue and Red spectral regions, and, therefore, several studies focused on the effect of Red and Blue lights on crop yield and quality (Kataoka et al., 2003; Li and Kubota, 2009; Pennisi et al., 2019a, Pennisi et al., 2020). Blue light strongly affects leaf expansion and stomatal opening and frequently promotes the development of a more efficient photosynthetic apparatus than Red light (Savvides et al., 2012; Miao et al., 2016). Red light promotes seed germination and growth and may enhance chlorophyll accumulation (Fan et al., 2013; Tiansawat and Dalling, 2013). In addition, the Red:Far-Red ratio controls plant architecture, germination and flowering, although with different responses depending on plant species and growing conditions (Demotes-Mainard et al., 2016). To the best of our knowledge, only minimal research has investigated the effects of LED light applied during cultivation on the post-harvest quality of tomato fruit (Affandi et al., 2020; Affandi et al., 2021). However, supplemental LED light application may be an interesting pre-harvest factor influencing post-harvest quality to be evaluated, given the activation of photoreceptor responses affecting plant metabolism. Accordingly, the present research aims to evaluate the effects of pre-harvest supplemental LED light on post-harvest quality of greenhouse-grown tomatoes, considering commercial storage standards.

3.4.3 Materials and Methods

3.4.3.1 Pre-harvest growing conditions

Tomato plants (*Solanum lycopersicum* L. ‘Siranzo’; Rijk Zwaan, The Netherlands) were cultivated in a commercial greenhouse located at Mezzolara di Budrio, Bologna, Italy (44°34’49” N, 11°31’54” E), using a high-wire hydroponic system (Paucek et al., 2020). Seedlings in rockwool cubes were transplanted in rock wool slabs (Grodan Vital, Roermond, Netherlands) on January 13, 2020. Plants were grown with a two-stem V-system with a planting density of 3.1 stems m^{-2}. The environmental conditions (temperature, relative humidity, solar radiation) were monitored daily during the entire growing period. A passive (lateral and top openings) and active (horizontal fan system) ventilation, as well as residual hot water flowing in tubes coming from an adjacent biogas system, were used to maintain the proper climatic conditions (T_{max} 37°C - T_{min} 11°C; RH_{max} 97% – RH_{min} 36%). A computer-controlled drip-irrigation system managed fertigation, maintaining the nutrient solution at average pH=6.0 and 2.6 dS m^{-1} average electrical conductivity (EC). The nutritive solution adopted by the commercial greenhouse is reported in **Table 10**. The supplemental lighting was provided by a single LED interlighting lamps (Flygrow Interlight, Flytech LED Technology, Belluno, Italy), located at 30 cm of distance from the stem, at the height of 1.40 cm from the rock wool slabs throughout the whole growing period. Three different lighting regimes were applied:

1. Red (660 nm) and Blue (465 nm) light with a Red:Blue ratio (R:B) of 3, a photosynthetic photon flux density (PPFD) of 180 µmol m^{-2} s^{-1} (measured at 30 cm from the plant) and a photoperiod of 16 h d^{-1} (8.00-00.00) (namely RB);
2. RB treatment with an addition of 44 µmol m^{-2} s^{-1} of Far-Red light (730 nm) during the whole photoperiod (namely FR);
3. RB treatment with an addition of 44 µmol m^{-2} s^{-1} of Far-Red, applied only for 30 minutes right after the 16 h d^{-1} of RB treatment (end-of-day) (namely EOD).

Control plants (CK) grown under natural light were also considered. Lighting treatments were applied only for the final phase of the productive farm cycle, from August until November 2020. A randomized block design was used with three blocks containing 7 plants per treatment (21 plants per treatment in total) (**Figure 22**).

Table 10. Nutritive solution adopted by the commercial greenhouse.

Main Component	Unit	Quantity
N-NO_3^-	mM	21.1
N-NH_4^+	mM	2.04
P-$H_2PO_4^-$	mM	1.56
SO_4	mM	3.15
H_3O^+	mM	8.34
K	mM	10.8
Ca	mM	8.05
Mg	mM	3.40
Na	mM	3.00
Cl	mM	1.00
Fe	µM	27.0
Mn	µM	12.0
Zn	µM	6.0
B	µM	20.0
Mo	µM	1.00

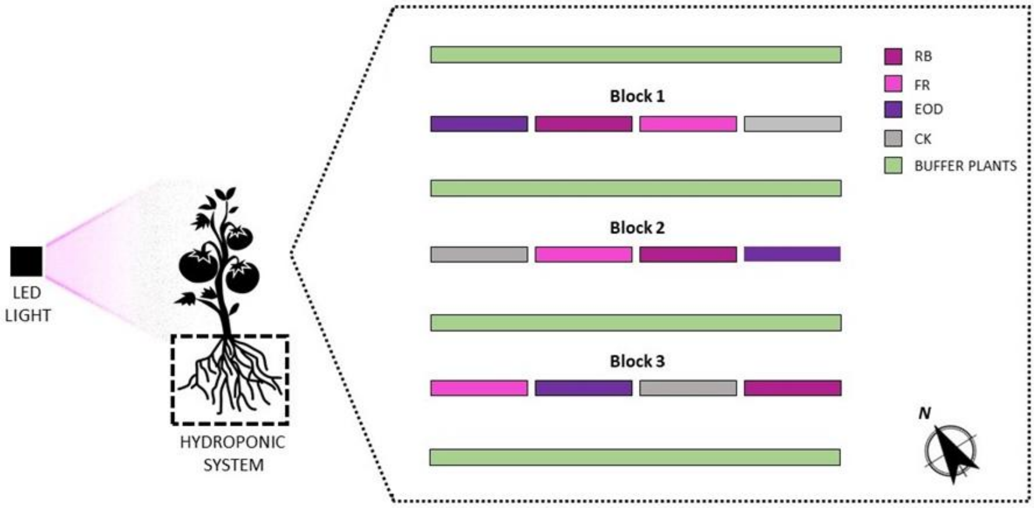


Figure 22. Experimental design of pre-harvest treatment (RB = Red:Blue; FR = Red:Blue + Fra-Red; EOD = Red:Blue + Fra-Red end-of-day; CK = Control plants).

3.4.3.2 Post-harvest preservation

Trusses at equal development stage were harvested from each plant. Afterward, fruit at red-mature stage and of the same size were selected among the harvested trusses. The fruit's red-mature ripening level was estimated by using a portable DA-Meter (DA-Meter, SINTELEIA srl, Bologna, Italy) (Rahman et al., 2019). DA-Meter is a portable device based on visible/Near Infra-Red (vis/NIR) spectroscopy developed to non-destructively assess fruit maturity (Farneti et al., 2015). Tomato fruits were selected for a uniform maturation red-ripe stage corresponding to a DA-index (I_{AD}) between 1.50 and 1.90. After the selection, tomatoes were immediately washed with sodium hypochlorite and stored in the dark at 13°C, with 80% relative humidity, for 7 days. The research applied one week of preservation basing on the standard of storage applied by the commercial greenhouse that furnished the tomatoes.

3.4.3.3 Weight loss and hardness

Weight loss was calculated on 6 fruits per treatment per block as the difference between the weight of the fruit at the beginning of storage (T0) and their final weight after 7 d of storage (T7) divided by the T0. To normalize data, weight loss values were expressed as % of the initial value. On the same fruits in the same days, fruit hardness was evaluated using a Durofel device (Giraud Technologies, Cavaillon, France) (Planton, 1991), fitted with a 0.10 cm^2 probe, on four opposite sides of the equatorial diameter of each fruit per time point. The instrument non-destructively measured the elasticity of fruit exocarp, expressing it in a Durofel Index ranging from 0 to 100.

3.4.3.4 Color determination

Color was evaluated on 6 fruits per treatment per block at T0 and T7 on the same fruit by using a CIE Lab color space analysis, where L* component represents the lightness from black (0) to white (100), a* component is a value ranging from green (−) to red (+), and b* component is a value ranging blue (−) to yellow (+). A colorimeter (Chroma Meter CR-400, Minolta, Tokyo, Japan) was used to assess the values. The measures were performed on four opposite sides at the equatorial diameter fruit level. Two indexes, HUE angle (h) and Chroma (C), were deduced from a* and b* components applying the formulas $\tan^{-1} (b^*/a^*)^2$ and $(a^{*2} + b^{*2})^{0.5}$, respectively (Lopez Camelo and Gomez, 1998).

3.4.3.5 Destructive measurements for fruit quality evaluation

Destructive measurements were performed on 6 fruits per treatment per block, other than those of non-destructive measurements, and included pulp firmness, soluble solids content and titratable acidity evaluation. Pulp firmness was determined using a fruit texture analyzer (FTA GÜSS, Strand, South Africa), evaluating the force required to penetrate the fruit. The penetration was performed with a cylindrical and flat-end probe of 6 mm of diameter, with a depth equal to 11 mm and a speed of 30 mm s^{-1}. Measurements were performed on four opposite sides of the equatorial diameter, peeling the fruit before penetration. Soluble solids content was evaluated on each centrifuged fruit using a digital refractometer model PAL-1 (Atago Co., Ltd., Tokyo, Japan). Titratable acidity was measured with an automatic TitroMatic (Compact-S titrator, Crison, Modena, Italy), diluting 20 mL of tomato juice in 20 mL of distilled water. The titratable acidity was estimated by titrating with 0.1N NaOH until the titration end-point of pH 8.1.

3.4.3.6 Total polyphenols and total antioxidant capacity

Total polyphenols and total antioxidant capacity were analyzed on 4 tomatoes per treatment per block, other than those of non-destructive measurements. At T0 and T7, fruit samples were immersed in liquid nitrogen and kept at -80°C for analysis. Samples of 4 g of homogenized freeze-dried fruit were placed in tubes and 8 mL of extraction mixture (60% methanol, 30% H_2O, 10% acetone) were added (Hartmann et al., 2008). The extraction was carried out by centrifugation at 1677 g for 10 min at 4°C. The supernatant was collected and used for antioxidant and total phenols analysis.

Total antioxidant capacity was analyzed using the FRAP (Ferric Reducing Antioxidant Power) method, developed following the method described by Benzie and Strain (1999), applying slight modifications. A reaction mixture containing acetate buffer (pH 3.6), 300 mM, 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) solution (in 40 mM HCl) and 20 mM $FeCl_3$ was prepared in a v:v:v proportion of 10:1:1 and incubated for 2 h in darkness. Then, 1.2 mL of the reaction mixture was added to 20 μL of supernatant and incubated for 1 h at room temperature in darkness. The antioxidant capacity was referred to a 0-2.5 mM $FeSO_4$ calibration curve. Samples and standards were read at 593 nm with a spectrophotometer (Biochrom Ltd, Cambridge, England).

Total polyphenols were determined using the methodology described by Waterhouse (2002), applying slight modifications. Briefly, 50 µL of the sample extract was added to 800 µL of Folin-Ciocalteu reagent diluted 1:15 (v:v) in H_2O. Gallic acid calibration standards up to 400 µg mL^{-1} were also included in the test. After an incubation of 5 min, samples and standards were added with 150 µL of 20% Na_2CO_3, incubated for 1 h at room temperature and then read at 765 nm with a spectrophotometer (Biochrom Ltd, Cambridge, England). Results of total phenols and antioxidant activity were expressed in gallic acid and Fe^{2+} equivalents, respectively, on a fresh weight basis.

3.4.3.7 Lycopene and β-carotene content

Lycopene and β-carotene contents were evaluated on 4 tomatoes per treatment per block, the same as total polyphenols and total antioxidant capacity measurement, using the methodology described by Anthon and Barrett (2006), applying slight modifications. An extraction solution was prepared by mixing hexane, acetone and ethanol in a v:v:v proportion of 2:1:1, plus 0.5 g L^{-1} of butylated hydroxytoluene. Briefly, 0.5 g of homogenized frozen sample, including exocarp and mesocarp, were mixed with 10 mL of extraction solution. The material was left in darkness for 30 min and then centrifuged at 2000 × *g* for 5 min. Finally, 1 mL of supernatant was read at 503 and 444 nm with a spectrophotometer (Biochrom Ltd, Cambridge, England).

The lycopene content was calculated using the following formula (Anthon and Barrett, 2006):

$$\mathrm{lycopene}\left(\frac{\mathrm{mg}}{\mathrm{kg}}\right) = \left(\frac{\mathrm{X}}{\mathrm{Y}}\right) \times \mathrm{A}_{503} \times 3.12$$

where X is the volume of hexane phase (mL, see below), Y the weight of the fruit tissue (g), A_{503} is the absorbance at 503 nm, and 3.12 is the extinction coefficient. β-carotene was calculated with the following equation (Anthon and Barrett, 2006):

$$\beta\text{- carotene} = (9.38 \times A_{444} - 6.70 \times A_{503}) \times 0.55 \times 537 \times \frac{V}{W}$$

where A_{444} is the absorbance at 444 nm, A_{503} is the absorbance at 503 nm, 0.55 is the ratio of the final hexane layer volume to the volume of mixed solvents added for hexane:acetone:ethanol (2:1:1), V is the volume of mixed solvents, W is the fresh weight of the sample, and 537 (g mol^{-1}) is the molecular weights of lycopene and β-carotene.

3.4.3.8 Statistical analysis

Non-destructive measurements (weight loss, fruit hardness, color) were analyzed through a repeated measures one way-ANOVA. Destructive measurements (pulp firmness, soluble solids content and titratable acidity) and biochemical evaluations were analyzed through a one-way ANOVA, by comparing lighting regimes within the different time (T0 and T7). Tukey's test was used for means comparison. Data were analyzed by using SPSS software.

3.4.4 Results

No differences in weight loss (as % from initial fruit weight) were observed between lighting treatments (mean weight loss of 1.8%, **Table 11**). The evaluation of fruit exocarp hardness showed no differences among treatments at T0. However, CK fruits were softer after one week compared to RB and FR ones (**Figure 23a**). The same trends were observed in flesh firmness evaluation, although no significant differences were observed among treatments at both measured times (T0 and T7) (**Figure 23b**).

Table 11. Mean values ± SD of qualitative parameters not reporting statistical difference ($p < 0.05$) among treatments, at harvest (T0) and after one week of storage (T7).

	CK		**RB**		**FR**		**EOD**	
	T0	T7	T0	T7	T0	T7	T0	T7
Destructive analysis								
Soluble solids (%)	4.3±0.3	3.9±0.2	4.3±0.2	4.1±0.3	4.0±0.3	4.0±0.3	4.2±0.1	4.1±0.3
Acidity (g L^{-1})	3.9±0.6	3.4±0.3	4.3±0.3	3.3±0.3	4.1±0.5	3.4±0.4	4.2±0.1	3.8±0.2
Non-destructive analysis								
*L**	41.4±0.8	40.4±1.1	41.7±0.6	40.3±1.0	41.7±1.8	40.8±1.3	42.5±1.3	40.9±1.6
*a**	23.1±0.7	21.0±1.8	25.2±1.6	23.5±1.6	23.8±1.6	22.3±2	24.2±1.9	22.4±2.2
*b**	30.5±0.9	28.5±1.0	31.1±0.9	30.2±1.0	30.2±1.5	28.8±1.5	30.9±1.7	30.0±1.4
h	0.9±0.02	0.9±0.03	0.9±0.02	0.9±0.02	0.9±0.01	0.9±0.03	0.9±0.03	0.9±0.03
C	38.3±0.9	35.4±1.8	40.0±1.6	38.3±1.7	38.5±2.1	36.5±2.3	39.3±2.3	37.4±2.3
*Weight loss (%)**		2.2±0.7		1.8±0.9		1.6±0.6		1.4±1.4
Biochemical analysis								
Phenols (GA 100 g^{-1})	29.0±5.3	26.0±2.8	24.9±2.3	26.5±1.5	32.19±5.0	26.7±2.3	26.0±2.5	24.5±2.2
Antoxidant activity (mmol Fe^{2+} 100 g^{-1})	0.3±0.1	0.3±0.4	0.2±0.05	0.3±0.02	0.3±0.03	0.3±0.03	0.3±0.05	0.3±0.05

*Considering the difference between T0 and T7

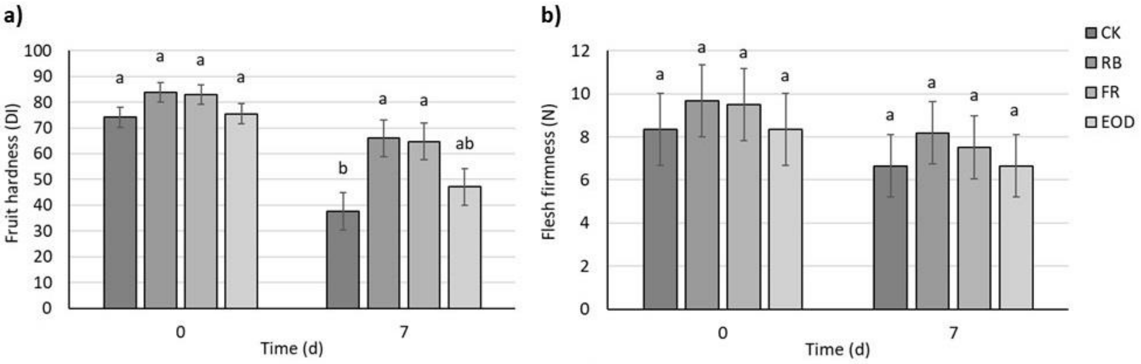


Figure 23. (a) Fruit hardness (Durofel Index, DI) after 0 and 7 d of storage ($p < 0.05$). TIME*TREATMENT: F(3;12)=2.609, p=0.010. **(b)** Flesh firmness (N) after 0 and 7 d of storage ($p < 0.05$). In charts, vertical bars indicate SD.

Evaluations of fruit color did not show any difference among treatments neither at harvest nor after 7 days of storage (**Table 11**). Mean fruit values at T0 and T7 were respectively 24.1 and 22.3 (a*), 30.7 and 29.4 (b*), 41.8 and 40.6 (L*), 0.91 and 0.92 (h), 39.0 and 36.9 (C). Similarly, soluble solid content (average value T0: 4.2 and T7: 4%) and titratable acidity (average value of T0: 4.1 and T7: 3.4 g L^{-1}) were not significantly influenced by lighting treatments (**Table 11**).

Biochemical analysis showed no differences among treatments in the case of total phenols content (average value 28.2 and 25.9 mg GA eqs. 100 g^{-1}) and antioxidant activity (average values of 0.28 and 0.28 mmol Fe^{2+} eqs. 100 g^{-1}), at T0 and T7, respectively (**Table 11**). Instead, carotenoid analysis demonstrated a significantly lower level of lycopene and β-carotene in fruits grown with natural light only (CK) compared to fruits grown under RB light treatment, after one week of post-harvest storage (**Figure 24a** and **Figure 24b**).

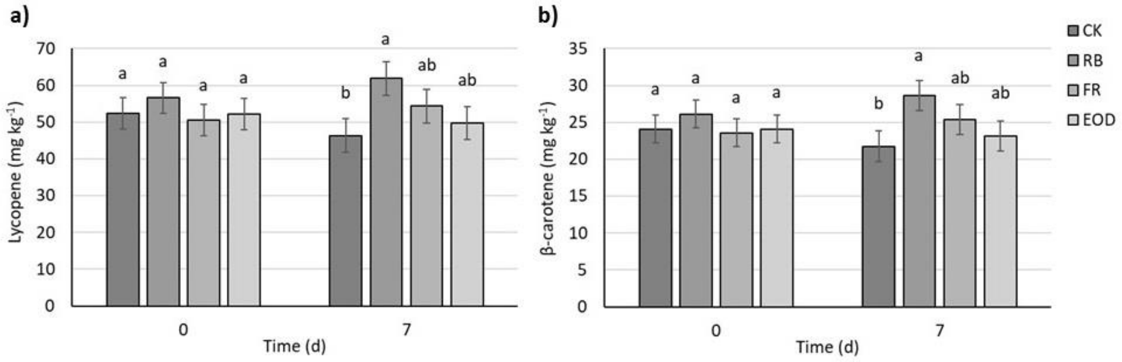


Figure 24. (a) Lycopene content (mg kg^{-1}) after 0 and 7 d of storage ($p < 0.05$), (n=8). **(b)** β-carotene content (mg kg^{-1}) after 0 and 7 d of storage ($p < 0.05$), (n=8). In charts, vertical bars indicate SD.

3.4.5 Discussions

Weight loss is one of the main problems of quality reduction in horticultural products during post-harvest (Nassarawa et al., 2021). The reasons for weight loss of tomatoes during storage can be attributed to environmental conditions, such as fruit's dehydration (Fagundes et al., 2015), as well as to normal cellular metabolic processes such as transpiration and respiration (Abiso et al., 2015). In the present research, tomatoes grown under different LED light treatments (RB, FR and EOD) or under natural light only (CK) did not show any significant difference in weight loss during storage (**Table 11**). Unfortunately, few researchers have investigated the effects of pre-harvest LED light on tomato post-harvest quality, often without reporting the weight loss effect. In Affandi et al. (2020), Far-Red light added to a Red and Blue base illumination has been found effective in reducing the relative weight loss of tomatoes during storage. The reduction in weight losses caused by Far-Red addition was related to an increase in cuticular thickness leading to a reduced transpiration rate (Cozmuta et al., 2016). Interestingly, in our experiments, all light treatments applied during cultivation resulted in a higher fruit skin hardness after 7 d of storage, suggesting that supplementary LED light may have increased cuticle thickness. This hypothesis is further corroborated by the fact that light treatments influenced only skin hardness but not flesh firmness. Light could affect skin hardness and flesh firmness differently, considering that the two instruments have different evaluation parameters. For example, by measuring the elasticity of the peel, Durofel index can be more influenced by the percentage fruit water content than the penetration evaluation.

The visual and sensorial parameters which have a more immediate effect on the consumers' perception and acceptance of the product are color, sweetness, acidity and consistency of the pulp. This research did not show any significant variation between treatments (**Table 11**), neither at the time of harvesting nor after one week of storage, in any of the commercial quality and organoleptic parameters described above. At the beginning of the storage, fruits used for the experiments were selected at the same ripening level using the DA-Meter. Accordingly, they did not present any color difference among treatments, both for color parameters (a*, b* and L*) and for color indices (h and C). After one week, lighting regimes did not affect colors attributes. The absence of color changes at the end of the storage period of ripe red tomatoes grown under supplemental LED light was already observed by the previous research on the effects of pre-harvest LED light on stored tomatoes by Affandi et al (2021). However, the same research observed a reduction in NAI (Normalized Anthocyanin Index) values as the cold storage period continued, highlighting a possible process of pigment degradation (Farneti et al., 2012). Regarding fruit sweetness and acidity, the absence of differences at harvest time was already observed in previous research (Dzakovich et al., 2015; Dzakovich et al., 2017; Paucek et al., 2020). Several harvest and post-harvest factors affecting fruit metabolic processes can influence the taste and flavor of tomato fruit during storage (Žnidarčič et al., 2010; Beckles, 2012). In our case, the absence of differences in soluble solids and acidity among treatments after one week of storage may be imputed to the advanced ripening stage at harvesting time, having reached the higher amount of storable sugars in fruit while still attached to the plant (Arias et al., 2000; Carrari et al., 2006), without incurring in soluble solids respiration and consequent reduction during one week of storage (Kader, 1987; Sualeh et al., 2016).

The results of the analysis of antioxidant capacity and total phenols content did not show significant differences between treatments, either at the beginning or at the end of the storage period (**Table 11).** However, it is well known that light composition affects the expression of genes that modulate the synthesis of secondary metabolites, including phenolic compounds, although such effects may depend on specific wavelength and/or plant species (Gupta, 2017; Baenas et al., 2021; Appolloni et al., 2022a). Concerning the antioxidant capacity, several authors have reported an increase in response to the application of LED lighting during the storage of tomatoes, either when a UV or Red and Blue LED light was used (Liu et al., 2011; Baenas et al., 2021). However, several other factors contribute to fruit’s antioxidant capacity, including climatic factors and ripeness (Valiulina et al., 2015), which may have masked the light-induced increase in antioxidant capacity. In fact, overripe fruit tends to lose antioxidant capacity compared to unripe (Valiulina et al., 2015), probably masking a possible effect among the different treatments in our case. Palmitessa et al. (2021b) reported an increase in antioxidant capacity, specifically for the lipophilic fraction, also in freshly harvested tomatoes grown with supplemental LED light. Notably, lycopene has been described as a potent antioxidant and the most active in the organic phase against free radicals (Cano et al., 2003; Baenas et al., 2021), and its content after the storage is highest in RB-treated samples and lowest in CK ones. The absence of differences in antioxidant capacity in our analysis could be associated with the hydrophilic extraction adopted in our protocol (Srivastava and Srivastava, 2015). Concerning total phenols content, both studies that directly applied LED light to tomatoes during post-harvest (Kokali et al., 2016; Baenas et al., 2021) and additional LED light in pre-harvest without post-storage evaluations (Dzakovich et al., 2017), would seem to confirm little effect on these compounds. However, the lack of significant differences among treatments at T0 and T7 in our research might be driven by a too short storage time (Bravo et al., 2012; Liu et al., 2012; Baenas et al., 2021), which could have limited the differences among treatments also in terms of decay. The present research decided to apply a standard commercial storage of one week as practiced by the farm. However, future research might consider more extended storage periods, using time as a factor to statistically confirm observations. Finally, the differential results from existing literature could also be associated with a different response to the lighting treatment associated with genotypic determinants (Mditshwa et al., 2017).

Carotenoid biosynthesis appears to be stimulated by Red and Blue LED light due to modulation of gene expression and light receptors (Mditshwa et al., 2017; Baenas et al., 2021). In particular, phytochrome appears to be the light receptor most involved in lycopene synthesis, being observed that Red light treatment can increase lycopene content in green-mature tomatoes by 2.3-fold (Alba et al., 2000). In our research, it was observed that lycopene content at harvest time did not differ significantly between treatments. This observation seems to contrast with the former hypothesis that associates LED light applied during pre-harvest with increases in the lycopene content in tomatoes (Ngcobo et al., 2020; Dannehl et al., 2021), although our observation may be related to the uniformity of the fruits used for the storage, all at an advanced stage of ripeness. However, after one week of storage, the treatments showed a significant difference in carotenoids (lycopene and β-carotene) content, particularly between CK grown under natural light only and the RB treatment (**Figure 24a** and **Figure 24b**). In the case of FR and EOD treatments, no significant differences were observed compared to CK, although the carotenoid content was still higher (**Figure 24a** and **Figure 24b**). An increase in red color of tomatoes is usually associated with an increase in carotenoids content (Carrillo-López and Yahia, 2014). In our case, the tomatoes used for the post-harvest measures were selected at an advanced mature-red stage, possibly having achieved the maximum synthesis of carotenoids. For this reason, it is possible to hypothesize that the differences observed at T7 are more attributable to a different decay time of carotenoids instead to the biosynthesis of new molecules. Indeed, it has been observed that lycopene in red tomatoes stored at 13°C tends to undergo a decay process (Farneti et al., 2012).

3.4.6 Conclusions

The research demonstrated that supplementary LED lighting applied during the cultivation of greenhouse tomatoes might allow maintaining hardness and carotenoid content in mature-red tomatoes after a standard commercial storage period at 13°C for one week. The observed results on increased fruit hardness are of particular interest from the standpoint of post-harvest handling and transportation losses. A greater skin hardness, which may lead to lower transpiration, can have positive effects on the reduction of food waste due to mechanical damages and weight loss during post-harvest. The absence of alteration of organoleptic features, such as color, sweetness, acidity and consistency of the pulp, may represent a positive aspect for consumers, maintaining unchanged the habitual perception of the product and consequent its marketability. Finally, concerning nutritional value, the higher presence of carotenoids after one week in fruit belonging to plants exposed to LED lighting may be a sign of a slower decay of product compounds, with positive consequences on preserving nutritional properties throughout time. The results open to future perspectives concerning supplemental LED light application to reduce food losses and maintain nutritional traits during post-harvest storage. As preliminary research, future developments could focus on analysis considering a longer time factor, including tomatoes harvested in earlier stages of development than red-mature. Research should also develop toward the evaluation of other crops besides tomato, as well as cost-benefit analysis in terms of the economic value of food savings compared to costs of LED light application. Finally, since the application of LED light may only have an effect in light limiting scenarios, the evaluation of the effect conserving the same Daily Light Integral (DLI) - reducing sunlight with shade cloth in LED treatments, for instance - should also be considered.

3.5 Chapter 5: Evaluation of tomato seedlings growth response under different qualities of supplemental LED light

Elisa Appolloni[1], Andrea Strano[1], Ivan Paucek[1], Giuseppina Pennisi[1], Andrea Crepaldi[2], Francesco Orsini[1], Xavier Gabarrell[3], Giorgio Gianquinto[1]

[1]Alma Mater Studiorum – Università di Bologna, DISTAL – Department of Agricultural and Food Sciences, Bologna, Italy

[2]Flytech srl, Via dell'Artigianato, 65, 32016 Alpago, Belluno, Italy

[3]Universitat Autònoma de Barcelona, Institut de Ciència i Tecnologia Ambientals (ICTA-UAB), MdM Unit of Excellence; Chemical, Biological and Environmental Engineering Department, Barcelona, Spain

3.5.1 Abstract

Tomato seedlings (*Solanum lycopersicum cv. Siranzo*) were grown using supplemental LEDs (Light Emitting Diodes) light to evaluate growth, physiological and morphological responses. Plants were sown on peat in a glass-glazed greenhouse at in Bologna, Italy (44°29'38"N, 11°20'34"E). Two monthly experiments were performed during spring season: first experiment from March until April 2021 (Exp. I) and second experiment from May until June 2021 (Exp. II). Supplemental LED lighting treatments were applied from sowing and consisted in Red and Blue (RB) (Ratio=3), Red and Blue (Ratio=3) + Fra-Red (FR) the whole day, and Red and Blue (Ratio=3) + Far-Red at the end-of-day (EOD). All treatments were applied for 16 h d^{-1} (h 8-00) with an intensity of 180 µmol s^{-1} m^{-2}. Control seedlings (CK), grown under natural light only, were also considered. The results showed a variable growth and morpho-physiological response of seedlings depending on lighting treatment. In particular, RB and FR treatments demonstrated to improve plants compactness, contemporarily guaranteeing a good photosynthetic performance. Among treatments supplied with artificial lighting, EOD plants presented longer hypocotyls. Finally, CK plants presented longer hypocotyls, higher leaf area and lower chlorophyll content, possibly as a response to light shortage.

Keywords: Supplemental light, Light Emitting Diodes, Seedlings, *Solanum lycopersicum*, Growth index.

3.5.2 Introduction

Tomatoes are one of the most cultivated crops worldwide, covering about 5 million hectares and with an overall production of 187 million tons in 2020 (FAOSTAT, 2022). To achieve such yield, the use of high quality seedlings is of fundamental importance, ensuring a better rooting and survival of plants after transplanting (Wei et al., 2018). Quality seedlings are often represented by green and compact plants, as excessive elongation may determine issues with fruiting and assimilates partitioning (Jeong et al., 2020). Furthermore, greater compactness may result in increased seedling tolerance to transport and transplanting into the final growing site (Kubota et al., 2004). However, in some cases, the production of seedlings with longer hypocotyls can be a favorable characteristic, as, for instance, in tomato grafting, a practice that can ensure optimal resistance to different types of stress, such as salinity, temperature, water, nutrient uptake, and diseases (Singh et al., 2017). In such case, long hypocotyls can be desirable to prevent scion exposure to soil and eventual pathogens, as well as to allow an easier grafting on rootstock (Chian and Kubota, 2010).

Light quality can play an important role in the tailored production of valuable seedlings. In particular, Red radiation (600-700 nm) can affect seedlings the growth and development of seedlings acting on photomorphogenesis (germination, hypocotyl inhibition), fresh weight, photomorphogenesis, as well as photosynthesis (fresh weight), as demonstrated by numerous studies on model plants (Sager and McFarlane, 1997; Kami et al., 2010). On the other hand, Blue light (400-500 nm) can also affect seedlings growth and development, including increased compactness, stomatal opening, photosynthesis, and phototropism (Kim et al., 2004; Kami et al., 2010). In general, Red and Blue light in a 3:1 ratio has been demonstrated to be the best combination to ensure optimal development and photosynthetic capacity of tomato seedlings (Liu et al., 2009; Son et al., 2018). However, Far-Red radiation (700-780 nm) appears to be the most suitable to obtain greater hypocotyl elongation, given the ability to trigger shade avoidance mechanisms in plants (Franklin and Whitelam, 2007). In particular, Far-Red at the end of the day, which simulates the natural light condition at dawn and dusk, seems to have a particular effect on stem elongation of different species including tomato (Blom et al., 1995; Decoteau and Friend, 1991; Kasperbauer and Peaslee, 1973).

The responses of plants to light are regulated by the photoreceptors, the molecules capable of absorbing specific wavelengths and triggering certain morpho-physiological responses. The main photoreceptors are phytochromes, cryptochromes and phototropins, which are responsible for the absorption of Red, Far-Red and Blue light (Kong and Okajima, 2016). In particular, the phytochrome is the photoreceptor mostly involved in stem elongation. This complex molecule is composed of two subunits, the chromophore and the apoprotein. The chromophore can undergo isomerization triggered by Red light absorption, resulting in the transition of the phytochrome from the Pr form, the original one, to the Pfr form, the active one. These two forms of phytochrome are said in dynamic equilibrium, which is dependent on the light quality absorbed and described through the photon flux ratio (R:FR). When the Pr form absorbs Red light, it converts into the Pfr state, on the contrary, when the Pfr form absorbs Far-Red light it returns to the Pr state. The ratio between Pfr/Pr+Pfr represents the photoequilibrium value (ϕ), according to which the plant perceives changes in light, triggering reaction signals such as stem elongation for shading avoidance (Runkle and Heins, 2001). The aim of the study was to evaluate the effects of different light spectra on the morphology, physiology and growth of tomato seedlings.

3.5.3 Materials and Methods

3.5.3.1 Growing conditions

Tomato seedlings (*Solanum lycopersicum* cv. *Siranzo*) (Rijk Zwaan Zaadteelt en Zaadhandel B.V, De Lier, Netherlands) were grown using supplemental LED (Light Emitting Diodes) light to evaluate the photomorphogenetic and physiological responses to different wavelengths. Plants were sown in plastic pots (6 x 6 x 7 cm) on a mixture of peat and perlite (~3:1, volume:volume), in a glass-glazed greenhouse in Bologna, Italy (44°29’38”N, 11°20’34”E). Two different experiments were conducted during spring, from March until April 2021 (Exp. I), and from May until June 2021 (Exp. II), respectively. Light treatments were applied by interlighting LED lamps (Flytech S.r.l., Belluno, Italy) placed at 30 cm from the vegetative apex for the duration of the experiments, following plants growth. Supplemental lighting was applied since plant sowing. Buffer plants were used to reduce the environmental effect around the samples. Plants were irrigated by subirrigation, adding a soluble NPK-based mineral fertilizer in a 14:15:15 ratio, with the addition of Boron (B), Manganese (Mn) and Zinc (Zn), with a dilution of 1.0 g/l.

Lighting treatments consisted of: Red (λ=600-700 nm) and Blue (λ=400-500 nm) radiation in a ratio of 3:1, (RB); Red and Blue radiation in a ratio of 3:1 plus Far-Red (λ=700-780 nm) provided throughout the duration of the photoperiod, (FR); and Red and Blue radiation in a ratio of 3:1 plus Far-Red at the end of the day for 30 minutes after photoperiod, (EOD). In both cases (FR and EOD), Far-Red was provided with an intensity of 40 µmol s^{-1} m-^{2}. The photoperiod of supplementary illumination lasted 16 hours per day, from 8:00 to 24:00, with a light intensity of 180 µmol s^{-1} m^{-2} at 30 cm from the lamps. A control supplied with natural light only was also considered.

Climatic conditions, both inside and outside the greenhouse, were recorded continuously throughout the duration of the test. Carbon dioxide (CO_2) levels inside the greenhouse were kept equal to those outside, with values of 400±20 ppm. Brightness, recorded inside the greenhouse during daylight hours (05:00 to 20:00) was 137 W m^{-2}during Exp. I and 165 W m^{-2} during Exp. II. In Exp. I, the mean daily temperature recorded inside the greenhouse was 21±4°C and an average relative humidity (RH) of 47±14%. During Exp. II, the average temperature recorded inside the greenhouse was 24±3°C and an average relative humidity (RH) of 57±11%.

3.5.3.2 Measurements

Destructive sampling collection was conducted at regular three- and four-day intervals for three weeks (5 measurements). Six plants (n=6) were collected randomly from each of the four treatments at each measurement. Measurements consisted of: leaf fresh weight, leaf dry weight, leaf total area, stem fresh weight, stem dry weight, stem length, hypocotyl length and chlorophyll content. Samples were dried in oven at 80°C for 72h. Weight measurements were used to evaluate dry matter content (DMC), as the percentage of the ratio between plant (total, stem and leaf) dry mass and fresh mass (g g^{-1}). Analysis of chlorophyll content was conducted by means of a chlorophyll meter (SPAD 502 Plus, Konica Minolta, Inc., Chiyoda, Tokyo, Japan) used on the median portion of the leaf page of non-cotyledonal leaves chosen randomly (4 measurements for each plant). Leaf area was performed by manual separation of individual leaves from the plant and placement inside a photographic scanner (Epson Perfection v370, Seiko Epson Corporation, Suwa, Nagano Prefecture, Japan). The digitalized images of the leaf area were virtually isolated and calculated using two different photo editing softwares: Adobe Photoshop 2019 and ImageJ v. 1.8.0.

3.5.3.3 Growth indexes

The evaluated growth indexes were: Relative Growth Rate (RGR, g $g^{-1}d^{-1}$); Net Assimilation Rate (NAR, g $cm^{-2}d^{-1}$); Leaf Area Ratio (LAR, cm^2 g^{-1}); Leaf Weight Ratio (LWR, g g^{-1}); Specific Leaf Area (SLA, cm^2 g^{-1}).

The RGR represents the rate of dry mass production per unit total dry weight and time, expressed in g $g^{-1}d^{-1}$ (Williams, 1946). This index is calculated as follows:

$$\mathrm{RGR} = \frac{\overline{\ln \mathrm{W_2}} - \overline{\ln \mathrm{W_1}}}{\mathrm{t_2} - \mathrm{t_1}}$$

where W_1 and W_2 are the total dry weights of the plants at times t_1 and t_2. The NAR represents the rate of dry weight production per unit leaf area and time, expressed in g $cm^{-2}\cdot d^{-1}$ (Williams, 1946). This index was calculated as follows:

$$\mathrm{NAR} = \frac{\ln \mathrm{A_2} - \ln \mathrm{A_1}}{\mathrm{A_2} - \mathrm{A_1}} \cdot \frac{\mathrm{W_2} - \mathrm{W_1}}{\mathrm{t_2} - \mathrm{t_1}}$$

where A_1 and A_2 are the leaf area at t_1 and t_2. The LAR represents the ratio of leaf area to total dry weight, expressed in cm^2 g^{-1} (Radford, 1967). This index was calculated as follows:

$$\mathrm{LAR} = \frac{\mathrm{A_2} - \mathrm{A_1}}{\ln \mathrm{A_2} - \ln \mathrm{A_1}} \cdot \frac{\ln \mathrm{W_2} - \ln \mathrm{W_1}}{\mathrm{W_2} - \mathrm{W_1}}$$

The LWR represents the ratio of the dry weight of leaves to whole plant dry weight, expressed in g g^{-1} (Kvet, et al., 1971). This index was calculated as follows:

$$\mathrm{LWR} = \frac{\mathrm{L_2} - \mathrm{L_1}}{\ln \mathrm{L_2} - \ln \mathrm{L_1}} \cdot \frac{\ln \mathrm{W_2} - \ln \mathrm{W_1}}{\mathrm{W_2} - \mathrm{W_1}}$$

Where L_1 and L_2 are the leaf dry weights at t_1 and t_2. Finally, the SLA represents the ratio of leaf area to leaf dry weight, expressed in cm^2 g^{-1} (Kvet, et al., 1971). This index was calculated as follows:

$$\mathrm{SLA} = \frac{\mathrm{A_2} - \mathrm{A_1}}{\ln \mathrm{A_2} - \ln \mathrm{A_1}} \cdot \frac{\ln \mathrm{L_2} - \ln \mathrm{L_1}}{\mathrm{L_2} - \mathrm{L_1}}$$

3.5.3.4 Statistical analysis

The statistical analysis was performed trough a one-way ANOVA and Tukey HSD post hoc test ($p<0.05$), using the SPSS statistical processing software, version 25.0 (IBM: International Business Machines Corporation, Armonk, New York, USA).

3.5.4 Results and Discussions

Experimental results showed an ability of light quality to influence some growth parameters of tomato seedlings, albeit with different degree depending on the growing season (Exp. I or II) and days since sowing. After 33 days from sowing, hypocotyl elongation of plants grown under RB and FR treatments showed significantly reduced length compared to CK and EOD in both experiments (**Table 12**). In particular, the reduction in length was observed mainly in the RB treatment, where plant hypocotyls were about 20% and 40% smaller than CK in Exp. I and Exp. II, respectively. Stem elongation also showed the same trend as hypocotyl, although EOD presented a reduced length compared to control plants in the case of the late spring experiment (Exp. II) (**Table 12**).

Table 12. Hypocotyl length, stem length, leaf area, dry matter content and chlorophyll content of tomato seedlings after 33 days from sowing. Significant difference at $p \leq 0.05$ are indicated by different letters.

	Exp.	**Treatment**			
		CK	**RB**	**FR**	**EOD**
Hypocotyl length (cm)	I	5.57^{a}	4.47^{b}	4.13^{b}	5.22^{a}
	II	5.58^{a}	3.32^{b}	3.70^{b}	4.83^{a}
Stem length (cm)	I	12.07^{a}	8.98^{b}	9.07^{b}	11.97^{a}
	II	19.08^{a}	9.92^{c}	12.52^{bc}	14.80^{b}
Leaf area (cm^{2})	I	149.07^{a}	77.67^{c}	92.57^{bc}	107.97^{b}
	II	180.36^{a}	122.18^{b}	95.85^{b}	119.96^{b}
Total dry matter content (%)	I	7.97^{b}	13.85^{a}	12.19^{a}	11.66^{ab}
	II	6.53^{b}	13.37^{a}	12.41^{a}	13.53^{a}
Stem dry matter content (%)	I	5.55^{b}	7.93^{a}	7.52^{a}	4.80^{b}
	II	5.14^{b}	8.29^{a}	7.98^{a}	8.81^{a}
Leaf dry matter content (%)	I	10.70^{a}	17.72^{a}	14.5^{a}	16.10^{a}
	II	9.55^{b}	19.25^{a}	18.29^{a}	19.73^{a}
Chlorophyll content (Index)	I	31.78^{c}	43.75^{a}	47.77^{b}	42.63^{b}
	II	33.27^{c}	46.61^{a}	46.52^{a}	38.37^{b}

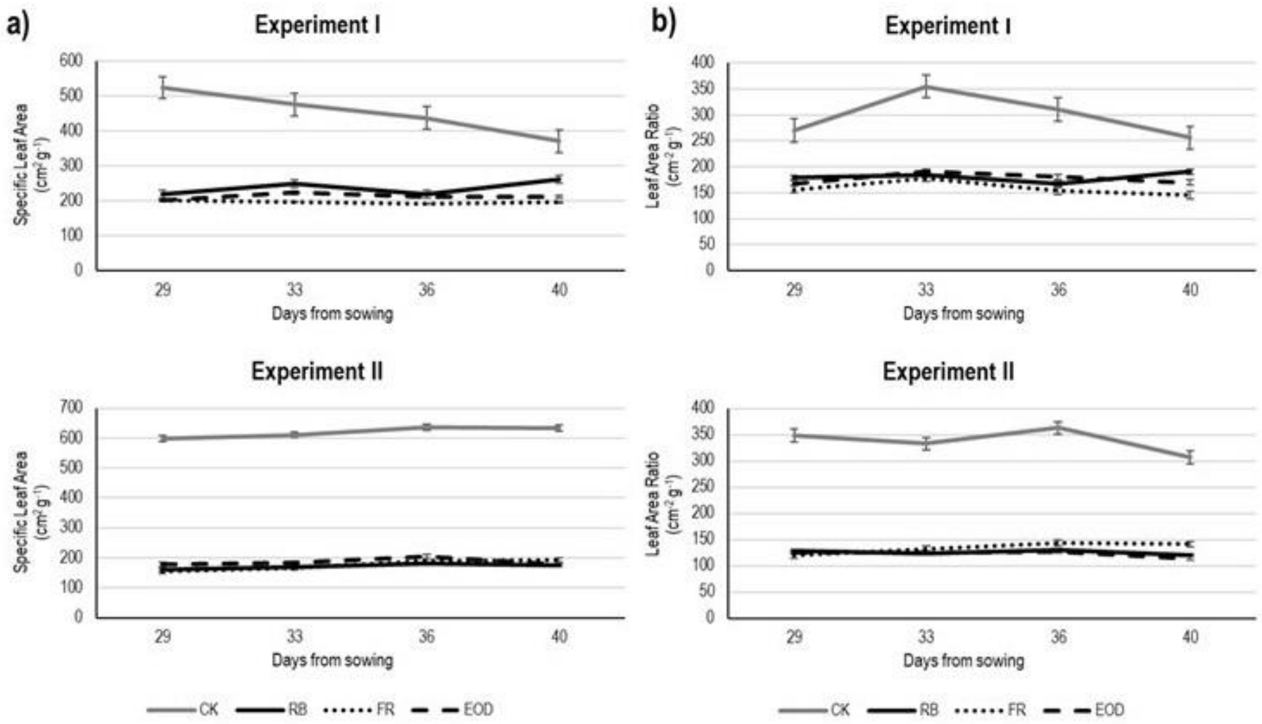
The increased length observed in the control can be related to plants attempt to escape mutual shading and move toward the light source. This phenomenon, also defined as "Shade Avoidance Syndrome" (Smith and Whitelam, 1997), is regulated by the phytochrome and, in particular, by the ratio between the Pfr and Ptot (Pfr + Pr), assuming a value between 0 and 1. As the ratio decreases, the length of the plant increases, given the higher presence of Far-Red light and the lower R:FR ratio (Runkle and Heins, 2001). This condition can occur in the case of very dense vegetation capable of transmitting or reflecting light. In fact, chlorophyll and carotenoids absorb the Red and Blue wavelengths, while reflecting the less absorbed radiations such as Green and Far-Red. The last is perceived by plants as a signal factor triggering the escape from shade (Holmes and Smith, 1977).

The presence of Red or Far-Red light can also undergo fluctuations according to weather and seasonal conditions, as well as according to the time of the day (Holmes and Smith, 1977). At dawn and dusk, the sun is lower and solar radiation travels a greater distance in the atmosphere, resulting in the dispersion of shorter wavelengths and higher presence of longer wavelengths. Therefore, the presence of Far-Red will be greater, triggering the above described mechanisms. In this research, such condition was simulated in the EOD treatment, in which a greater elongation than RB and FR treatment was observed, although still lower than in plants treated by CK. In EOD, this result can be attributed to the effect of the low R:FR ratio in the final 30 minutes of the photoperiod. On the contrary, the lower stem length compared the CK can be determined by a satisfactory light condition and consequent absence of shade avoidance, given the presence of additional light.

It is important to note that the lower elongation, in both RB and FR treatments, can be attributed not only to a high R:FR ratio throughout the photoperiod, but also to a synergistic dwarfing action of the Blue radiation known as “coaction” (Wollaeger and Runkle, 2014; Hernández, 2016). In addition to the phytochrome, also the cryptochrome, the photoreceptor absorbing Blue light, would seem to be responsible for the shortening phenomenon (Ahmad, 2002). Confirming this theory, Ninu et al. (1999) reported that the knock-out of the gene responsible for cryptochrome 1 synthesis significantly increased stem length in tomato, while Giliberto et al. (2005) observed that the overexpression of another gene responsible for cryptochrome 2 synthesis resulted in a significant decrease in stem length.

Regarding leaf area, plants grown under LED treatments showed reduced values by 48%, 38%, and 28%, in Exp. I, and by 32%, 47%, and 33%, in Exp. II, compared with the control plants, for RB, FR, and EOD treatments, respectively (**Table 12**). These observations appear to be in contrast to reports from previous studies in which the combination of Red and Blue (Hernández et al., 2016), as well as the presence of Far-Red (Kubota et al., 2012), would appear to be associated with increased leaf area. The absence of pronounced leaf expansion can be attributed to the relatively low level of Blue ubiquitously used within all light treatments. Indeed, recent experimental evidence has shown that high ratios of Blue light, between 50% and 75%, can expand leaf area and accelerate dry matter production, in contrast to lower levels close to 25%, which can partially inhibit tomato seedlings growth (Liu, et al., 2018). However, a higher leaf area in CK can also be related with light shortage and consequent surface expansion attempting to increase interception (Pettigrew et al., 1993; Pauli et al., 2017).

Specific Leaf Area (SLA) is an index of leaf thickness to which photosynthetic capacity is correlated (Reich et al., 1998). Analyses show significant variation in case of additional LED treatments compared to the control plants in both trials (**Figure 25a**). In particular, leaves under LED light resulted on average thicker by 52% and 71% for Exp. I and II, respectively, as compared to CK. On the contrary, Leaf Area Ratio (LAR), which provides a measure of the leaf ability to intercept light, was significantly greater in CK leaves probably as a response to contrast light shortage, with 42% and 62% higher surface than LED treatments in Exp. I and II, respectively (**Figures 25b**).



EXPERIMENT I

Days from sowing

	29		33		36		40	
CK	524.19	a	476.32	a	436.91	a	370.88	a
RB	219.49	b	249.02	b	219.47	b	261.24	b
FR	200.52	b	196.06	b	191.10	b	195.28	b
EOD	202.12	b	223.07	b	211.49	b	211.00	b

EXPERIMENT II

Days from sowing

	29		33		36		40	
CK	598.03	a	611.24	a	636.45	a	633.30	a
RB	160.55	b	168.26	b	181.52	b	176.20	b
FR	155.62	b	167.44	b	188.50	b	191.36	b
EOD	177.61	b	183.18	b	204.48	b	178.33	b

EXPERIMENT I

Days from sowing

	29		33		36		40	
CK	270.31	a	354.58	a	310.79	a	255.90	a
RB	179.03	ab	184.13	b	168.08	b	190.34	b
FR	156.49	b	177.91	b	153.73	b	145.64	b
EOD	167.84	ab	190.31	b	180.62	b	169.42	b

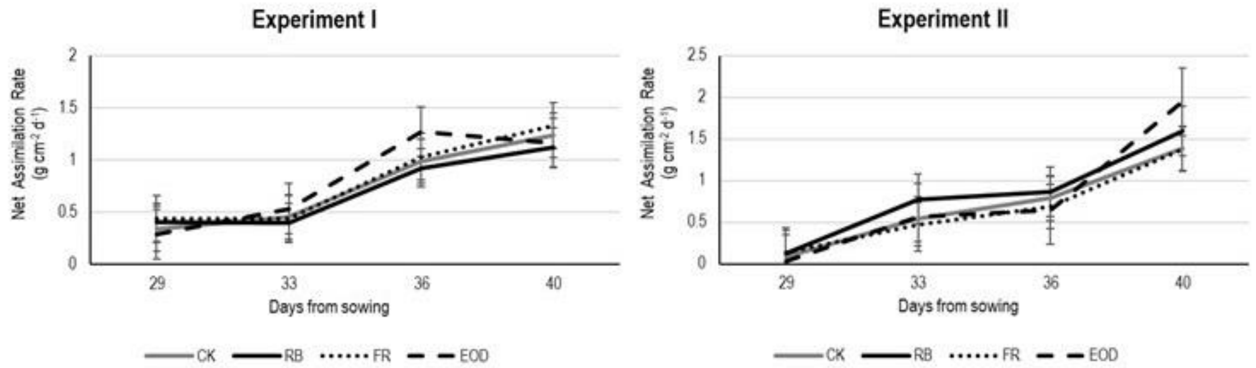
EXPERIMENT II

Days from sowing

	29		33		36		40	
CK	348.43	a	332.88	a	362.72	a	307.03	a
RB	128.22	b	123.72	b	130.26	b	120.76	bc
FR	119.83	b	131.85	b	143.70	b	141.15	b
EOD	125.82	b	125.29	b	127.20	b	113.85	c

Figure 25. Effects of different supplemental light treatments on Specific Leaf Area (SLA) **(a)** and Leaf Area Ratio (LAR) **(b)** recorded during Exp. I and Exp. II.

The above results may suggest that plants under LED treatment present a better photosynthetic rate and biomass production. In fact, a lower thickness and significant expansion of leaf area determines a reduction of mesophyll cells and thinning of the parenchyma tissue, resulting in a reduced number of chloroplasts per cell, lower carboxylation efficiency, and lower activity of Rubisco (Bauer and Thöni, 1988; Gonçalves, et al., 2008; Matos, et al., 2009). The observation can be confirmed by the results relative to the content of chlorophyll, which is localized in the chloroplasts of the palisade parenchyma cells (Jensen, 2007). Indeed, plants grown under supplemental LED light showed an overall significant increase in chlorophyll content compared to the control (**Table 12**), translatable to a total average of 34% and 26% higher value in Exp. I and II, respectively. The cause of the increase in chlorophyll content in tomato seedlings can be attributed to the action of Blue radiation, a wavelength necessary for normal chloroplast structure, as well as for regular leaf anatomy (Liu, et al., 2011). In addition, recent studies have shown that combined Red and Blue light treatments can have a better effect on chlorophyll content of tomato seedlings as compared to monochromatic light (Hernández et al., 2016). Nevertheless, some sources report how this phenomenon is not always observable, suggesting a response based on the genotypic characteristics of the cultivar (Liu, et al., 2011; Wollaeger and Runkle, 2014). Despite a higher chlorophyll content, the Net Assimilation Rate (NAR) did not show a significantly different trends among treatments (**Figure 26**). This result may mean that, even though leaf area and total dry weight presented differences influenced by the treatment, the excess of photosynthesis rate of the leaves over the respiration rate of the whole plants remained unchanged (Watson and Hayashi, 1965).



EXPERIMENT I

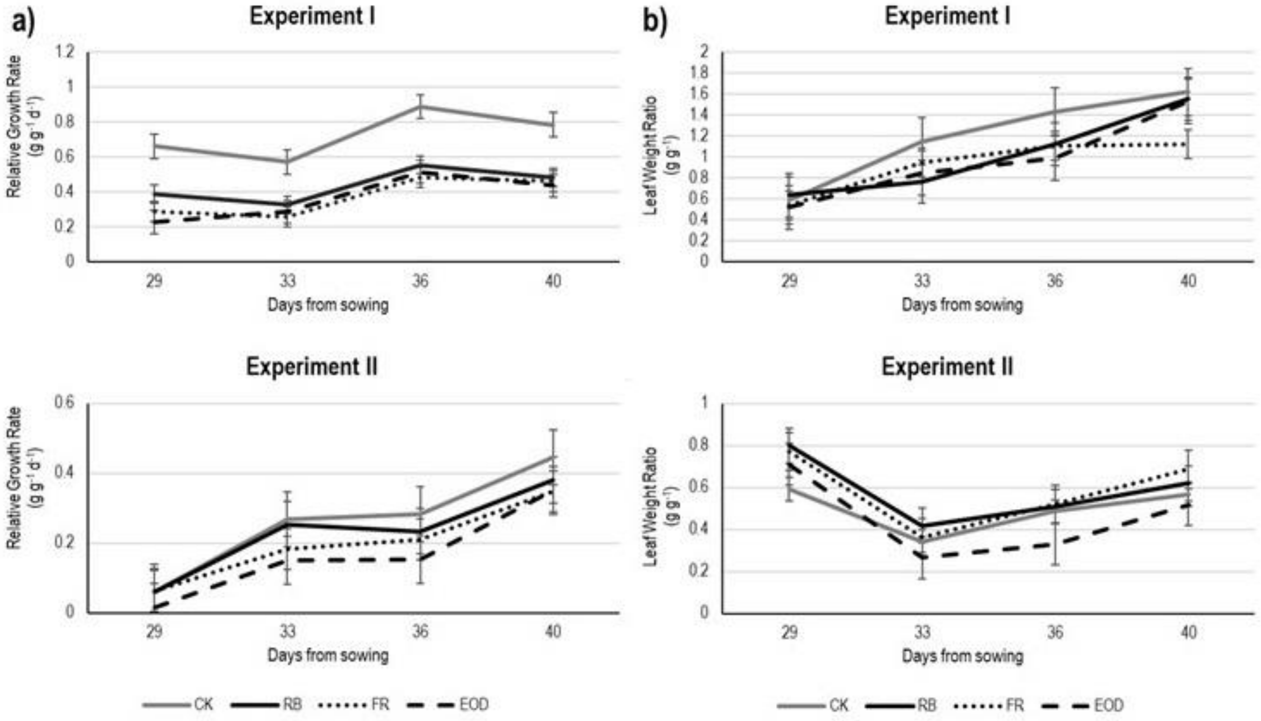
	Days from sowing							
	29		33		36		40	
CK	0.34	a	0.45	a	0.99	a	1.24	a
RB	0.40	a	0.40	a	0.92	a	1.12	a
FR	0.44	a	0.44	a	1.03	a	1.33	ab
EOD	0.29	a	0.53	a	1.27	a	1.16	b

EXPERIMENT II

	Days from sowing							
	29		33		36		40	
CK	0.08	a	0.54	a	0.79	a	1.38	a
RB	0.13	a	0.78	a	0.87	a	1.60	a
FR	0.14	a	0.47	a	0.69	a	1.38	a
EOD	0.03	a	0.56	a	0.64	a	1.95	a

Figure 26. Effects of different supplemental light treatments on Net Assimilation Rate (NAR) during Exp. I and Exp. II.

LED treated plants showed a higher content of dry matter contents as compared to the plants grown under natural light only (CK) (**Table 12**). Previous studies have shown the ability of artificial radiation, especially in a Red:Blue=3 combination, to increase dry matter content of tomato seedlings (Hernández et al., 2016). In the present research, small shifts of tendency seem to occur depending on growing period (Exp. I and II) and days after sowing (data not shown), being probably related not only to an effect of the additional light, but also to the different environmental conditions (natural light and temperature) taking place. These changes in trends probably influenced by different environmental conditions, can also be observed in the Relative Growth Rate (RGR) index, in which the CK plants showed significantly higher values in the early spring experiment (Exp. 1), while no significant differences in the late spring experiment (Exp. 2) (**Figure 27a**). On the contrary, the Leaf Weight Ratio (LWR) did not present significantly different trends between treatments, showing a homogeneous distribution among the sink organs of the sampled plants (**Figure 27b**).



EXPERIMENT I

	Days from sowing			
	29	33	36	40
CK	0.66 a	0.57 a	0.89 a	0.79 a
RB	0.39 b	0.33 b	0.56 b	0.48 b
FR	0.29 b	0.26 b	0.48 b	0.46 b
EOD	0.23 b	0.29 b	0.52 b	0.44 b

EXPERIMENT II

	Days from sowing			
	29	33	36	40
CK	0.06 a	0.27 a	0.28 a	0.45 a
RB	0.06 a	0.25 a	0.23 ab	0.38 ab
FR	0.06 a	0.18 ab	0.21 bc	0.35 b
EOD	0.01 a	0.15 b	0.15 c	0.35 b

EXPERIMENT I

	Days from sowing			
	29	33	36	40
CK	0.59 ab	1.15 a	1.43 ab	1.62 ab
RB	0.63 a	0.76 a	1.12 ab	1.56 ab
FR	0.54 ab	0.94 a	1.11 a	1.13 a
EOD	0.52 b	0.84 b	2.12 b	1.53 b

EXPERIMENT II

	Days from sowing			
	29	33	36	40
CK	0.59 c	0.34 ab	0.49 a	0.57 ab
RB	0.80 a	0.42 a	0.51 a	0.62 ab
FR	0.77 ab	0.36 ab	0.52 a	0.69 a
EOD	0.71 b	0.27 b	0.33 b	0.52 b

Figure 27. Effects of different supplemental light treatments on Relative Growth Rate (RGR) **(a)** and Leaf Weight Ratio (LWR) **(b)** during Exp. I and Exp. II.

3.5.5 Conclusions

The results of the research showed an ability of the RB and FR treatments to increase plant compactness by shortening the stem and hypocotyl and reducing leaf expansion. These effects did not occur as effectively on plants subjected to EOD treatment, where the low R:FR ratio at the end of the day induced a slight, though appreciable, elongation of the plant's epigeal organs.

It is possible to state that the supplementary RB light represents the most valid lighting treatment for the production of more compact and resistant tomato seedlings with satisfactory photosynthetic performance. The EOD treatment, as reported by other authors, led to a greater elongation of the stem and hypocotyl. Since the variety used in the experiment is not a rootstock variety, but rather a genotype adapt to direct transplantation, the result obtained may be unsuitable for direct transplant. However it would be interesting to validate the effects of EOD treatment for rootstocks production in appropriate genotypes of tomato.

Tomato cultivars can respond very differently to the same lighting treatments, highlighting a highly significant genotypic-specific component. Future research should focus on the genotypic response of different tomato cultivars to light components, as well as considering an extension of the research to the fruiting stage, to understand the effects that additional light at the beginning of plant development may have on the distribution of assimilates.

3.6 Chapter 6: Winter greenhouse tomato cultivation: matching supplementary lighting and leaf pruning for improved yield and precocity

Elisa Appolloni[1], Ivan Paucek[1], Luigi Manfrini[1], Giuseppina Pennisi[1], Xavier Gabarrell[2], Francesco Orsini[1], Giorgio Gianquinto[1]

[1] Alma Mater Studiorum – Università di Bologna, DISTAL – Department of Agricultural and Food Sciences, Bologna, Italy

[2]Universitat Autònoma de Barcelona, Institut de Ciència i Tecnologia Ambientals (ICTA-UAB), MdM Unit of Excellence; Chemical, Biological and Environmental Engineering Department, Barcelona, Spain

3.6.1 Abstract

The solar radiation entering a high-wire tomato greenhouse is mostly intercepted by the top of the crop canopy, while the role of lower leaves diminishes with age, turning them into sink organs rather than sources. Accordingly, defoliation of basal leaves is a widely applied agronomic practice in high-wire greenhouse cultivation management. However, the recent increase in the application of supplemental LED (Light Emitting Diode) lighting for high-density tomato production may affect the role of basal leaves, promoting their source role for fruit development and growth. The present research explores the application of supplementary LED lighting on *Solanum lycopersicum* cv. Siranzo in the Mediterranean area during the cold season, in combination with two regimes of basal defoliation: early removal of the leaves (R) right under the developing truss before fruit turning stage, and a non-removal (NR) during the entire cultivation cycle. The lighting factor consisted of an artificial LED lighting treatment with Red and Blue diodes for 16 h d^{-1} (h 8-00) with an intensity of 180 µmol s^{-1} m^{-2} (RB), and a control cultivated under natural light only (CK). The results showed a great effect of supplemental LED light increasing total yield (+118%), favoring fruit setting (+46%) and faster ripening (+60%), regardless defoliation regimes, although the increased energy prices hindered the economic viability of the technology.

Keywords: Light Emitting Diode (LEDs), Greenhouse, Defoliation, *Solanum lycopersicum*.

3.6.2 Introduction

Defoliation is an agronomic practice widely used in tomato cultivation, consisting on the removal of leaves below a cluster before harvesting, at times and intensities often dependent on producer management choices. The objectives of defoliation include increasing aeration between plants, limiting disease development, promoting illumination of lower clusters, and facilitating cultivation operations (Heuvelink; 2018). Basal leaf removal may also affect the yield, reducing the sink effect of less illuminated leaves in the lower canopy and promoting the arrival of assimilates to developing fruits (Jo and Shin; 2020).

The link between defoliation and yield is determined by the translocation and partitioning of assimilates between plant organs. Translocation refers to the movement of photo-assimilates from the producing organs, or sources, to the recipient organs, or sinks, while partitioning refers to the proportion by which assimilates are allocated to the various sinks (Osorio et al., 2014; Anuradha and Bishnoi, 2017). The two processes described are crucial in determining the accumulation of dry matter in fruits, therefore affecting the final yield (Ronga et al. 2017). The division of organs into source and sink is not always obvious since one organ can take on both roles (Aslani et al., 2020). Moreover, each organ possesses a certain sink strength, being more or less capable of attracting the products of photosynthesis due to physical (e.g., number of cells) and molecular (e.g., enzymes activity) features (Bihmidine et al., 2013; Li et al., 2021b).

Cultivation density, and consequently vertical light distribution, can affect leaves sink-source role. Research showed that under conditions of high planting density (around 30 cm spacing on the row) basal leaves seems to experience strong competition for light, contributing little to the plants net photosynthesis and becoming sink organs, while larger spacing (around 50 cm on the row) can significantly contribute to assimilates production making defoliation before harvest not favorable for fruit development (Hachmann et al., 2014). Highly dense cultivation is the norm in high tech greenhouses, where the use of supplemental LED interlighting has already been demonstrated to be capable of increasing yield and other quality aspects of greenhouse tomato production, affecting plant photosynthesis and plants photoreceptors responses (Ouzounis et al., 2015). The present research aims at evaluating if the application of two defoliation regimes, namely removal at fruit turning stage and non-removal, combined with two lighting regimes, namely supplemental LED light treatment and control grown with natural light, can significantly affect the role of basal leaves and, consequently, yield and quality of tomato production.

3.6.3 Materials and Methods

3.6.3.1 Plants growing conditions and treatments

Tomato plants (*Solanum lycopersicum* L. cv. Siranzo; Rijk Zwaan, The Netherlands) were cultivated in a glass-glazed greenhouse in Bologna, Italy (44°29’38”N, 11°20’34”E). The seedlings were produced in rockwool cubes (Grodan Vital, Roermond, The Netherlands) by a local nursery from mid-August and were transplanted in perlite bags at a distance of 20 cm on the row on September 23rd, 2021. The environmental conditions (temperature, relative humidity, solar radiation) were daily monitored during the entire growing period. Both passive (lateral and top openings) and active (fan and heat pump) climate control strategies were used to maintain constant conditions (T_{mean} 22°C; RH_{mean} 64%). Fertigation was performed by an open drip-irrigation system, providing a solution with average pH of 6.6 and electrical conductivity (EC) of 2.8 dS m^{-1} (**Table 13**). Supplemental lighting was provided by single LED interlighting lamps (Flygrow Interlight, Flytech LED Technology, Belluno, Italy), located at 30 cm of distance from the stem, at a height of 1.40 cm from the rockwool slabs throughout the whole growing period. Two lighting regimes were applied: a control illuminated with natural light only (namely CK), and an illuminated treatment supplied with Red (660 nm) and Blue (465 nm) LED lights (RB ratio of 3), with a photosynthetic photon flux density (PPFD) of 180 µmol s^{-1} m^{-2} (measured at 30 cm from the plant) and a photoperiod of 16 h d^{-1} (namely RB). Lighting treatments were applied from transplanting day (September 23rd, 2021) until the end of the experiment (February 16th, 2022). Since the beginning of November, the plants have undergone two defoliation regimes either consisting in an early removal of the leaves right under the developing truss before fruit turning point (R), and a non-removal during entire cultivation cycle (NR). Accordingly, the experiment accounted for a total of four treatments, in a factorial design that included LED light + defoliation (RB+R), LED light + non-defoliation (RB+NR), natural light + defoliation (CK+R), and natural light + non-defoliation (CK+NR). A randomized block design with four blocks was used, including 5 plants per individual block.

Table 13. Formulation of standard nutrient solution used for tomato cultivation.

	Unit	Nutrient solution
$N\text{-}NO_3$	mmol L^{-1}	14.00
$N\text{-}NH_4$	mmol L^{-1}	1.00
P	mmol L^{-1}	1.00
K	mmol L^{-1}	8.00
Ca	mmol L^{-1}	4.00
Mg	mmol L^{-1}	1.50
Na	mmol L^{-1}	0.00
$S\text{-}SO_4$	mmol L^{-1}	2.50
Cl	µmol L^{-1}	0.00
Fe	µmol L^{-1}	15.0
B	µmol L^{-1}	20.0
Cu	µmol L^{-1}	1.0
Zn	µmol L^{-1}	5.0
Mn	µmol L^{-1}	10.0
Mo	µmol L^{-1}	1.0

3.6.3.2 Plant vegetative, physiological and biochemical measurements

Collar diameter was measured once at the end of October, at height of 1 cm from the rockwool cube. Internodes length was measured at the end of October as the distance among fourth and fifth fruit trusses. Plants topping occurred on November 23rd, above the sixth truss. Total leaf area was evaluated at the end of the experiment by using a leaf area meter (LI-3100C Area Meter, LI-COR Biosciences, Lincoln, United States) on NR plants, cultivated with RB or CK treatment. Leaves and stems were weighted with a digital scale, fresh and after being dried at 60°C per 4 days. Weight measurements were used to evaluate leaf dry matter content (LDMC), as the ratio between leaf dry mass and leaf fresh mass (mg g^{-1}), and specific leaf area (SLA), as the ratio between leaf area and leaf dry mass (m^2 kg^{-1}) (Garnier et al., 2001).

Chlorophyll content of leaves was evaluated at mid-December, considering two points of the first leaf right under the third and fourth fruit truss. A SPAD-502PLUS (Konica Minolta, Tokyo, Japan) was used to non-disruptively estimate the chlorophyll content. Leaf gas exchange and chlorophyll fluorescence were evaluated using a portable LI-COR 6400 (LI-COR Biosciences, Lincoln, United States), set as reported by Calone et al. (2021). In particular, the following parameters were measured: stomatal conductance (GS, in mmol m^{-2} s^{-1}), under-leaf CO_2 concentration (Ci), leaf transpiration (E, in mmol m^{-2} s^{-1}), net photosynthesis (A, in µmol m^{-2} s^{-1}), and effective quantum yield efficiency of PSII (ΦPSII). The ΦPSII, which represents the capacity of photosystem II (PSII) to absorb photon energy, has been calculated as (Fm'-Fs)/Fm', where Fm' represents the maximum fluorescence in a leaf adapted to light, and Fs represents the steady state fluorescence. The ΦPSII has been further split in two components: the PSII maximum efficiency (Fv'/Fm') and the level of photochemical quenching of PSII (qP). The first component (Fv'/Fm') represents the maximum operational efficiency in a leaf adapted to light, and is calculated as (Fm'—Fo')/Fm', where Fo' is the minimum fluorescence. The second component (qP) represents the real amount of active reaction centers of PSII, and is calculated as (Fm'-Fs)/(Fm'-Fo').

3.6.3.3 Fruit development and yield

Fruit development was monitored on proximal and distal fruit of both first cluster (Follow up 1) and third cluster (Follow up 2), by measuring the equatorial and polar diameters with a digital Vernier caliper before harvesting. The volume (mm^3) of the fruit was estimated as the volume of an ellipsoid of rotation $V=(4/3)\,\pi ab^2$, where a is one half of the polar diameter and b is one half of the equatorial diameter (Li et al., 2015). Fruit ripening was evaluated on the same fruit two weeks before harvesting by using a DA-Meter (Sinteleia, Bologna, Italy), which non-destructively evaluated the chlorophyll degradation and correlate it with a ripening index.

From the end of November until the end of the trial, fruits were harvested (in total, 6 clusters per plant). The fresh weight of total clusters of each plant in each treatment was measured with a digital scale. The number of fruits per cluster was counted for each plant. At harvesting, fruits were divided, counted and weighted as mature (dark orange and red tomato) and immature (green, light green and light orange) (Paucek et al., 2020). Fruit productive units (flowers and buds) were counted at the beginning of truss development and confronted with the number of fruits at harvesting to assess fruit setting.

3.6.3.4 Fruit quality

Non-destructive measurements

Tomatoes used for qualitative analysis (n=12) were selected considering a DA-Meter range index ranging 1.30 to 1.50. Fruit hardness was assessed by using a Durofel device (Giraud Technologies, Cavaillon, France) on four opposite sides of the equatorial diameter of each fruit per treatment per block. The instrument non-destructively measured the elasticity of fruit exocarp, expressing it in a Durofel Index ranging from 0 to 100.

Color was evaluated by using a CIE Lab color space analysis, where L* component represents the lightness from black (0) to white (100), a* component is a value ranging green (−) to red (+), and b* component is a value ranging blue (−) to yellow (+). A colorimeter (Chroma Meter CR-400, Minolta, Tokyo, Japan) was used to assess the values. The measures were performed on four opposite sides at equatorial level. Two indexes, HUE angle (h) and Chroma (C), were deduced from a* and b* components applying the formulas $\tan^{-1} (b^*/a^*)^2$ and $(a^{*2} + b^{*2})^{0.5}$, respectively (Lopez Camelo and Gomez, 1998).

Destructive measurements

Destructive measurements included pulp firmness, soluble solids content and titratable acidity evaluation. Pulp firmness was determined using a fruit texture analyzer (FTA GÜSS, Strand, South Africa), evaluating the force required to penetrate the fruit. The penetration was performed with a cylindrical and flat-end probe of 6 mm of diameter, with a depth equal to 11 mm and a speed of 30 mm s^{-1}. Measurements were performed on four opposite sides of the equatorial diameter, peeling the fruit before penetration. Soluble solids content was evaluated on each centrifuged fruit using a digital refractometer model PAL-1 (Atago Co., Ltd., Tokyo, Japan). Titratable acidity was measured with an automatic TitroMatic (Compact-S titrator, Crison, Modena, Italy), diluting 20 mL of tomato juice in 20 mL of distilled water. The titratable acidity was estimated by titrating with 0.1N NaOH until the titration end-point of pH 8.1.

Fruit dry matter content (FDMC) was evaluated on 4 fruits per treatment per block, others than those used for destructive and non-destructive qualitative analysis, as the ratio between fruits fresh weight and dried weight at 65°C per 1 week.

3.6.3.5 Biochemical analysis

Lycopene and β-carotene content

Lycopene and β-carotene content were evaluated on 6 fruits per treatment per block using the methodology described by Anthon and Barrett (2006), applying slight modifications. An extraction solution was prepared by mixing hexane, acetone and ethanol in a v:v:v proportion of 2:1:1, plus 0.5 g L^{-1} of butylated hydroxytoluene. Then, 0.5 g of homogenized frozen sample, including exocarp and mesocarp, were mixed with 10 mL of extraction solution. The material was left in darkness for 30 min and then centrifuged at 2000 × *g* for 5 min. Finally, 1 mL of supernatant was read at 503 and 444 nm with a spectrophotometer (Biochrom Ltd, Cambridge, England).

The lycopene content was calculated using the following formula (Anthon and Barrett, 2006):

$$\mathrm{lycopene}\left(\frac{\mathrm{mg}}{\mathrm{kg}}\right) = \left(\frac{\mathrm{X}}{\mathrm{Y}}\right) \times \mathrm{A}_{503} \times 3.12$$

where X is the volume of hexane phase (ml, sew below), Y the weight of the fruit tissue (g), A_{503} is the absorbance at 503 nm, and 3.12 is the extinction coefficient. β-carotene was calculated with the following equation (Anthon and Barrett, 2006):

$$\beta\text{-}carotene = (9.38 \times A_{444} - 6.70 \times A_{503}) \times 0.55 \times 537 \times \frac{V}{W}$$

where A_{444} is the absorbance at 444 nm, A_{503} is the absorbance at 503 nm, 0.55 is the ratio of the final hexane layer volume to the volume of mixed solvents added for hexane:acetone:ethanol (2:1:1), V is the volume of mixed solvents, W is the fresh weight of the sample, and 537 (g mol^{-1}) is the molecular weights of lycopene and β-carotene.

3.6.3.6 Energy cost assessment

The energy cost assessment was made considering the actual consumption of a lamp with Red (660 nm) and Blue (465 nm) light in a ratio of 3 (1.68 kWh) applied for 16 hours per day. Costs were estimated per plant per day, considering that a single lamp was able to provide supplementary lighting to about 6 plants. Cost per plant was then used to calculate the cost per kg. The price of electricity was acquired from EUROSTAT (2021) dataset, considering electricity prices for non-household consumers (excluding VAT and other recoverable taxes and levies) in Italy, referring to both second semester of 2021 (0.1853 € kWh^{-1}) and first semester of 2022 (0.2525 € kWh^{-1}). For the final electricity cost calculation, considering the growing cycle of 147 days, the price of the second semester of 2021 was used for 100 days (from September 23rd 2021 to December 31st 2021), and the price of the first semester of 2022 for 47 days (from January 1st 2022 to February 16th 2022).

3.6.3.7 Statistical analysis

Data analysis was performed with a two-way ANOVA, by comparing lighting factor with the defoliation factor. Data were analyzed by using IBM SPSS Statistics 28.0.1.0.

3.6.4 Results

Evaluation of vegetative parameters showed a significant increase in the collar diameter of plants grown with supplementary LED light (+34%) (RB 13.1 ± 2.2 mm, CK 9.8 ± 1.5 mm), regardless the type of defoliation (data not shown). On the contrary, internode length, total leaf area, leaf fresh and dry weight, LDMC, plant total fresh and dry weight, and steam length did not show any statistical difference among treatments (data not shown). However, the measurement of SLA resulted in a significant increase (+41%) in case of CK (181 ± 21 cm^2 g^{-1}) treatment as compared to RB (128 ± 19 cm^2 g^{-1}) (data not shown).

Physiological response evaluation considered several parameters. Significant differences were, however, only observed in case of chlorophyll content (SPAD Index), net photosynthesis (A), effective quantum yield efficiency of PSII (ΦPSII), photochemical quenching of PSII (qP), and leaf transpiration (E). Particularly, SPAD Index resulted significantly higher in plants under RB treatment, both in leaves under third (+23%) and fourth (+17%) truss, independently from the defoliation regime (**Figure 28**). Similarly, net photosynthesis (A) was higher in plants exposed to RB treatment as compare to CK, but was not altered by the defoliation regime (**Table 14**). On the other hand, ΦPSII, qP and E showed significant differences not only among light treatments but also among defoliations regimes, reporting higher levels in case of RB light treatment and leaf removal (R) (**Table 14**), although not showing an interaction among factors. PSII maximum efficiency (Fv/Fm), stomatal conductance (GS), and under-leaf CO_2 concentration (Ci) were not affected by lighting and defoliation factors (data not shown).

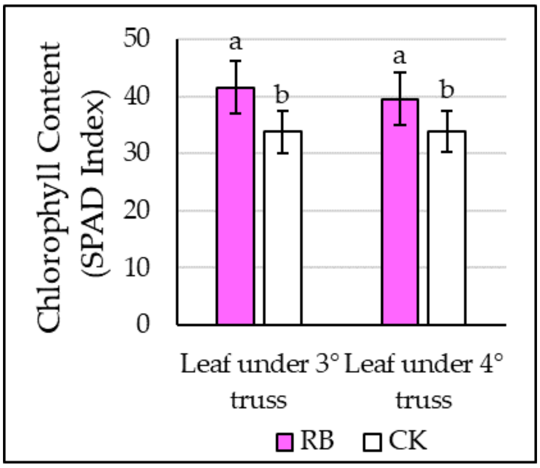


Figure 28. Effects of supplemental Red and Blue LED light (RB) and natural light only (CK) on leaves chlorophyll content (SPAD Index).

Table 14. Effects of the two separated factors, light (supplemental Red and Blue LED light, RB, and natural light, CK) and defoliation (early leaf removal, R, and no-removal, NR), on net photosynthesis (A), effective quantum yield efficiency of PSII (ΦPSII), photochemical quenching of PSII (qP), and leaf transpiration (E).

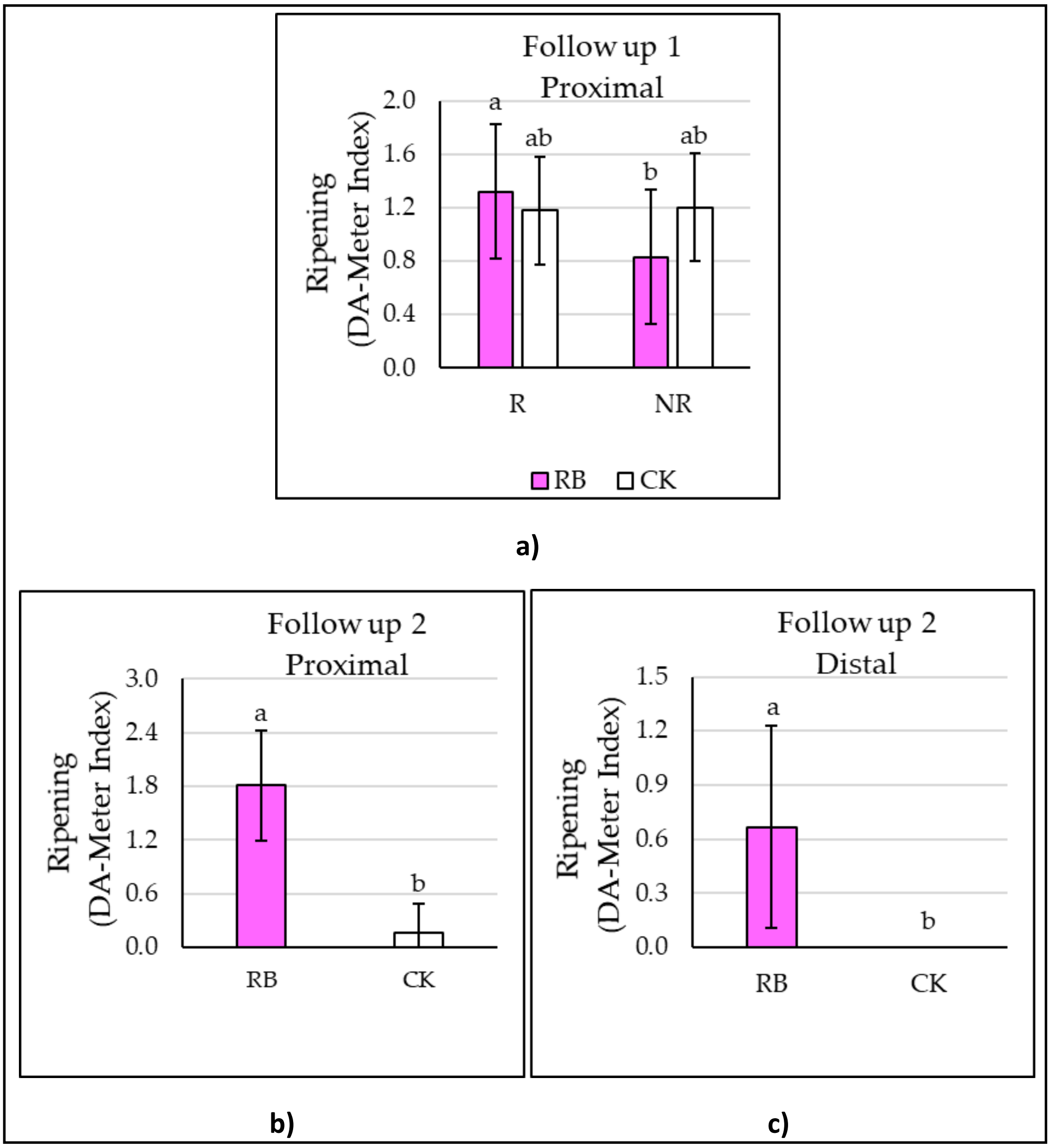
		A	ΦPSII	qP	E
Light	*RB*	6.7 ± 1.5^a	0.4 ± 0.1^a	0.5 ± 0.1^a	47.2 ± 11.3^a
	CK	5.1 ± 1.7^b	0.3 ± 0.1^b	0.4 ± 0.2^b	34.7 ± 14.2^b
Defoliation	*R*	6.4 ± 1.7^a	0.4 ± 0.1^a	0.5 ± 0.1^a	47.6 ± 12.5^a
	NR	5.4 ± 1.7^a	0.3 ± 0.1^b	0.4 ± 0.1^b	34.3 ± 12.7^b

In Follow-up 1, the volume of proximal fruit resulted significantly higher in case of plants subjected to defoliation (R), but not to light factor (**Table 15**). On the contrary, distal fruit of Follow-up 1 resulted significantly bigger in case of RB treatment as compare to CK, but were not significantly influenced by defoliation (**Table 15**). In case of fruit of Follow-up 2, the presence of supplemental RB light seems to significantly affect fruit dimension in both proximal and distal fruit, while defoliation regime did not show any significant effect (**Table 15**). Ripening evaluation of proximal fruit of Follow-up 1, showed significant higher values in case of early defoliation (R), also reposting an interaction effect with supplemental RB light (**Figure 29**). On the other hand, distal fruit of Follow-up 1 did not present any significant difference for both factors (**Figure 29**). During Follow-up 2, RB light significantly increased DA-Meter values both in proximal and distal fruit (**Figure 29**). In general, the RB treatment presented +60% higher ripening values as compared to CK, two weeks before harvesting.

In Follow up 1, the volume of proximal fruit resulted significantly higher in case of plants subjected to defoliation (R), while it was not affected by the lighting regime (**Table 15**). On the contrary, distal fruit of Follow up 1 resulted significantly bigger in case of RB treatment as compared to CK, but were not significantly influenced by defoliation (**Table 15**). During the Follow up 2, the presence of supplemental RB light significantly affected fruit dimension in both proximal and distal fruit, while defoliation regime did not cause any significant effect (**Table 15**). A significant interaction between lighting and defoliation factors was observed for ripening (measured by DA-Meter values) of proximal fruit during Follow up 1. Indeed, ripening of proximal fruit was increased by early defoliation (R) in RB treated plants as compared to NR plants, while no effects were observed in CK plants (**Figure 29a**). Conversely, ripening was not affected by either lighting or defoliation in distal fruit during Follow up 1 (data not shown). During Follow up 2, RB light significantly increased DA-Meter values both in proximal and distal fruit (**Figure 29b**, **29c**). In general, two weeks before harvesting, the RB treatment resulted in a higher rate (+60%) of ripened fruits as compared to CK.

Table 15. Effects of the two separated factors, light (supplemental Red and Blue LED light, RB, and natural light, CK) and defoliation (early leaf removal, R, and no-removal, NR), on fruit volume (cm^3) in proximal and distal tomatoes of Follow up 1 and Follow up 2.

		Follow-up 1		**Follow-up 2**	
		Proximal	*Distal*	*Proximal*	*Distal*
Light	*RB*	605.3±236.3^a	652.5±259.2^a	428.4±207.6^a	262.2±146.5^a
	CK	650.7±208.8^a	493.1±217.8^b	200.4±162.8^b	119.6±93.0^b
Defoliation	*R*	697.8±228.4^a	543.8±275.0^a	289.8±178.6^a	228.7±139.6^a
	NR	562.0±200.9^b	595.0±249.5^a	391.6±243.5^a	212.7±152.8^a



a)

b) **c)**

Figure 29. Effect of light (supplemental Red and Blue LED light, RB, and natural light only, CK) and defoliation (leaves early removal, R, and no-removal, NR) on **(a)** proximal fruits of Follow up 1, **(b)** proximal fruits of Follow up 2 and **(c)** distal fruits of Follow up 2. In case of ripening of proximal fruit of Follow up 1 **(a)** the factors of light and defoliation showed a significant interaction (p=0.015).

Total fruit yield resulted significantly increased in case of supplemental RB light application, with doubled production (+118%) as compared to CK (**Table 16**). LED light application also affected the yield of green and red tomatoes, as summarized in **Table 16**. Furthermore, differences were observed for the number of aborted and productive units (flowers and buds), respectively showing a decrease (-54%) in flowers abortions (RB 0.7±1.0, CK 1.6±1.5 of aborted units) and an increase

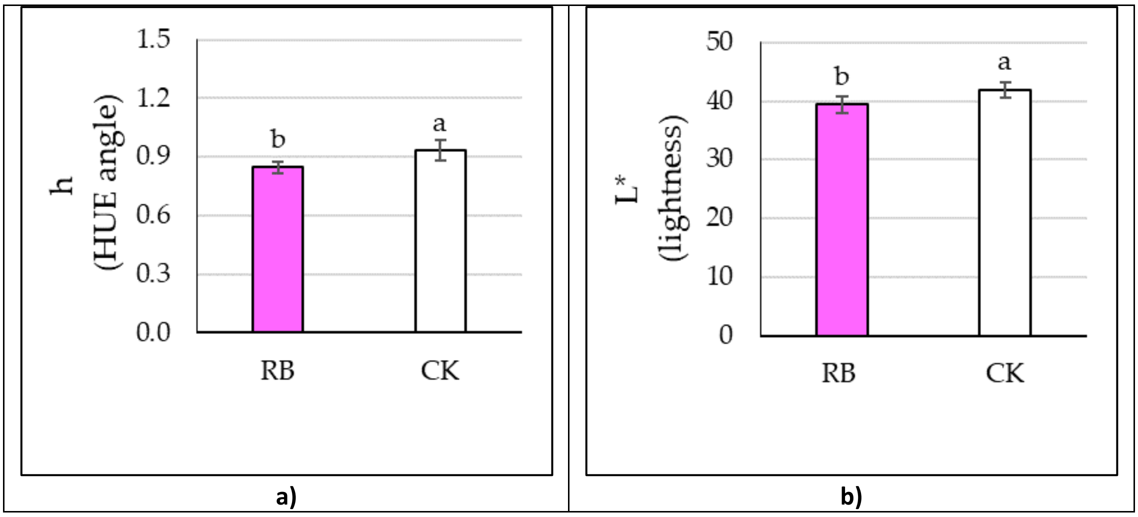
(+46%) in fruit setting (RB 6±1.5, CK 4.1±2.2 of set fruits) in case of RB light application, as compared to CK (data not shown). However, no significant differences were observed in case of the different defoliation regimes for yield, flower abortion and fruit setting.

Table 16. Effect of supplemental Red and Blue LED light (RB) and natural light only (CK) on tomato yield.

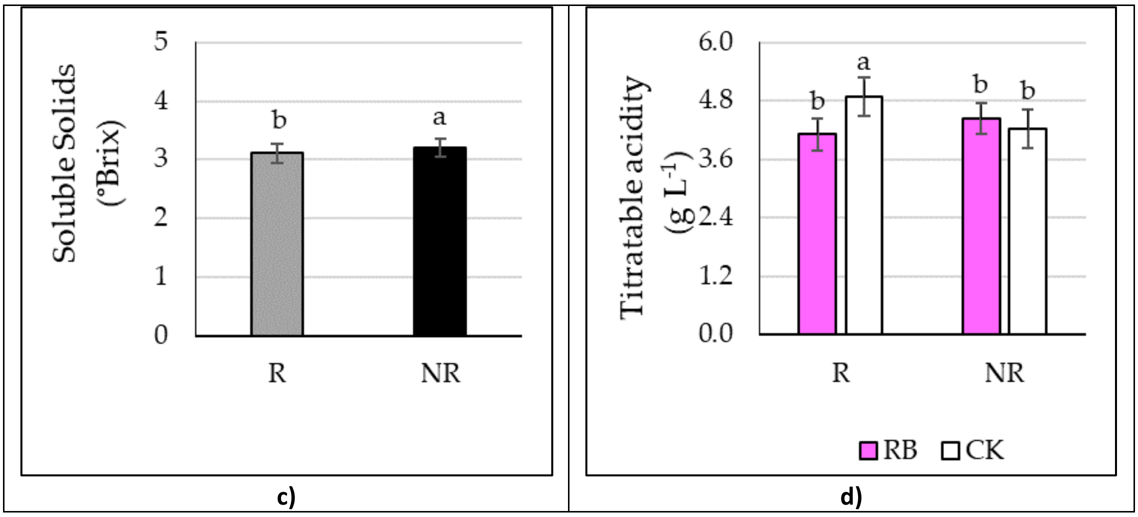
YIELD (g plant $^{-1}$)	**TOTAL FRUITS**	**RED FRUITS**	**GREEN FRUITS**
RB	3261.25±515^{a}	1980.15±479^{a}	624±249^{b}
CK	1495.7±305^{b}	127.8±193^{b}	843.8±260^{a}
Variation (%)	+118	+1449	-26

Fruit dry matter content (FDMC), Chroma (C), fruit hardness, and pulp firmness were not affected by both factors (data not shown). Among color determinations, HUE angle (h) and lightness (L*) presented significant higher levels in case of tomatoes grown under natural light only (CK) (**Figure 30a**, **30b**), while no significant differences were observed depending on defoliation regime. Soluble solids content showed statistically significant higher levels in case of tomatoes from plants not subjected to leaf removal (NR) (+3%) (**Figure 30c**), while no differences were observed depending on light treatment. A significant interaction between lighting and defoliation factors was observed for fruit acidity. Indeed, fruit acidity was increased by early defoliation (R) in control plants as compared to RB treated ones, while no differences between lighting regimes were observed in NR plants (**Figure 30d**).

Finally, none of the factors applied resulted in changes in either lycopene or β-carotene content (data not shown).



a) **b)**



c)

d)

Figure 30. Effect of light (supplemental Red and Blue LED light, RB, and natural light only, CK) and defoliation (leaves early removal, R, and no-removal, NR**)** on **(a)** HUE angle, **(b)** Lightness, **(c)** Soluble Solids and **(d)** Acidity of tomato fruits. In case of acidity **(d)**, the two factors of light and defoliation showed a significant interaction (p=0.000).

The energy consumption resulted around 1.68 kWh per day per lamp. Considering this consumption and the electricity costs (as reported in Section 2.6.), the total cost of supplemental LED lighting during the fall-winter period was about 8.51 € plant^{-1}, leading to 2.65 € kg^{-1}.

3.6.5 Discussion

Light is a fundamental factor for plant vegetative and architectural development. The effect of specific wavelengths on plant morphology are determined by the stimulation of photoreceptors, which perceiving a light spectrum can induce responses that ameliorate leaves exposition to solar radiation and, consequently, photosynthesis (Li et al., 2012; Dierck et al., 2017). This is particularly evident in case of the so called "Shade Avoidance Syndrome", in which a low Red/Fra-Red ratio (R:FR) can induce phytochroms, the photoreceptors reading the red wavelengths, to promote plant elongation and escape sub-optimal lighting condition (Ballaré et l., 2017). Blue light can also affect plant morphology, favoring the leaf area expansion (Wang et al., 2015). In the present research, Red and Blue light limitedly influenced vegetative parameters, reporting a significant increase only in case of collar diameter and SLA. The same increase in tomato collar diameter in case of supplemental RB light application was already observed by Puacek et al., (2020). On the other hand, the significantly lower SLA value in RB, seems to confirm the common response of plants in case of high light intensity, in which leaf thickness, determined by increased palisade tissue, can serve as a protective response against excessive irradiation (Fan et al; 2013).

Regarding physiological parameters, the higher chlorophyll content (**Figure 28**) and, consequently, the higher net photosynthesis (**Table 14**) observed in leaves under supplemental RB light, can be associated to an action of phytochromes and cryptochromes, respectively stimulated by Red and

Blue light components (Bukhov et al., 1992). Leaf defoliation normally reduces water evapotranspiration (Balducci et al., 2020). However, in the present study an opposite an trend was noticed. Pervious research, observed that the reduction of source-sink ratio may reduce transpiration and increase efficiency of PSII in tomato plants, especially under limited nutritive conditions (Glanz-Idan and Wolf, 2020).

Defoliation reported an effect on fruit dimension only in case of proximal fruits of Follow-up 1, while no differences were observed for distal fruit and during Follow-up 2 (**Table 15**). On the contrary, supplemental LED light significantly influenced the development of both proximal and distal fruits during Follow-up 2 (**Table 15**). While in the case of fruits of Follow-up 1 the application of supplemental LED light started with inflorescences presenting proximal fruits already formed, the fruits of Follow-up 2 began their development when already under the light treatment. As observed in previous researches (Paponov et al., 2020; Appolloni et al, 2022b), the major dimension of RB fruits may be related to a major sink strength, in turn determined by a possible stimulation of LED light during the initial phase of cell division rather than during the enlargement phase.

As already observed by other authors (Paucek et al., 2020), fruit maturation precocity seemed to be significantly increased by LED light, showing more evident effects especially in case of later phases of autumn season (December - Follow-up 2). As for fruit dimension, leaf removal seemed to have a significant higher effect on ripening only in the earlier stages of production (Follow-up 1) and in case of proximal fruits (**Figure 29a**). Although these effects can be associated with a lower assimilates competition with other sink organs including lower leafs, the observation was only related to the first period of production, when outside lighting condition did not result excessively limited.

The qualitative characteristics of tomatoes, such as color, flavor and firmness, are of fundamental importance as they influence consumers' perception (Pinheiro et al., 2013). In the present research, supplemental LED light limitedly affected qualitative traits. Carotenoids content was often reported to increase in case of LED treatments, especially in case of Red light application, given a possible involvement of fruit phytochromes (Alba et al., 2000, Nájera et al., 2018; Dannehl et al., 2021). However, the absence of significant differences in the herby study could be related to a limited exposition of the fruit to the LED light, which did not directly stimulated carotenoids development. Defoliation determined a significant decrease in soluble solids content (**Figure 30c**), as a possible consequence of the imbalance between sink/source equilibrium (Casierra-Posada et al., 2013).

From an economic point of view, the additional electricity costs incurred when greenhouse artificial lighting is applied should be counterbalanced by the observed yield increase (+118 %). However, if an overall increase in costs associated to energy consumption could be estimated in 2.65 € kg^{-1}, when deducting from the calculation the achievable yield under solar radiation only, the actual costs per kg was even higher, namely 4.82 € kg^{-1}. Considering that producers price of vine tomato in January 2022 accounted for 1.82 € kg^{-1} in Italy (EC, 2022), the application of supplemental LED lighting was not proved. However, a number of concurrent factors may have affected these performances. From a cost perspective, energy costs were increased from January 2021 (when the experiment was designed) respectively by 15 and 37 % after 6 and 12 months. Although an extended artificial photoperiod was used (16 h d^{-1}) throughout the growing cycle, some authors suggested to avoid supplementary lighting when solar radiation is sufficient (Niu et al., 2022) or to specific phenological stages, to reduce energy consumption. On the other hand, the estimated income is linked to both agronomic yield and producer price. The short-term nature of the experiment may have hindered the final yield. The research was performed by top-pruning plants at the 6th truss instead of the at least 15-20 trusses commonly obtained from the same cultivar in local commercial greenhouses (Pauceck et al., 2020). Besides, the adopted cultivar, although adapted to high-wire intensive cultivation, is associated with low prices in the Italian market (e.g., about half the price of cherry tomatoes (EC,2022)).

3.6.6 Conclusions

Although defoliation is considered the best practice to guarantee easier management, favor ventilation and control pest development, the research revealed that this practice can limitedly influence tomato yield and quality in case of supplemental LED light application. The use of supplemental LED light demonstrated interesting potentialities for tomato production during the cold season in the Mediterranean countries, increasing total yield (+118%), favoring fruit setting (+46%) and fastening ripening (+60%). In relation to energy costs and measured yield, the use of supplemental lighting was however not proven economically feasible under the tested conditions. Accordingly, future studies should match energy saving strategies (e.g., reducing light integrals by disruptive lighting protocols) with further characterization of both genotypic and phenotypic responses to lighting.

4 DISCUSSIONS

4.1 Supplemental LED light to increase production in Mediterranean countries

In the present research particular attention was given to the evaluation of the potential of supplemental LED light in Mediterranean countries, in which the technological input for greenhouse production is still limited (Perdossi et al., 2018). Some researches have already stared to investigate the application of this technology in southern Europe, obtaining interesting results especially in terms of productive capacity. In particular, Paucek et al. (2020) observed that the application of supplemental Red and Blue light in an intensive commercial greenhouse during spring-summer period can increase the yield by 16% and accelerate fruit ripening as compared to a control cultivated with natural light alone. Palmietessa et al., (2020) observed the same capacity, increasing the yield by 22% with Red+White+Blue LED light, and opening to interesting scenarios of application for off-season production during December-March. In our case, the evaluation of productive capacity showed very similar trends for the spring-summer season in an i-RTG (**Exp. 1**), achieving 17% higher yield as compared to a control grown with natural light only. During winter production (**Exp. 4**), our results showed an even much higher total yield increase (+118%) as compared to Palmitessa et al. (2020a), remarking an interesting possible application of this technology for cold season production.

The results showed that the increase of yield might be related to a much higher number and higher dimension of fruits as compared to the control. In particular, during **Exp. 1** control tomatoes resulted 9.3% lighter and 7.2% fewer as compared with tomatoes grown under LED treatments. On one hand, the major dimension of LED treated tomatoes may be related to a higher sink capacity of fruit given by a possible stimulation of cell division during the first stage of fruit development (Paponov et al., 2020). On the other hand, the higher number of fruits could be related to a capacity of LED light to increase the number of flowers per inflorescence (Heuvelink et al., 2018; Palmitessa et al., 2020a), also affecting flowers allegation as showed during **Exp. 4**.

Despite the increase of productive capacity, some aspects still need to be considered. In particular, energy cost is the main aspect to take into account, as it can affect the economic feasibility of supplemental LED light application. During **Exp. 1**, the summer-spring production did not seem to be economically feasible given the high electricity cost as compared to sellable price. The same limitations were observed diring **Exp. 4**, where the recently grown electricity prices and the contained market value of vine-tomato revealed that cultivation with LED light may not be economically advantageous even during the winter period. However, a change of strategy in the management of the supplementary lighting could contribute to an improvement of the economic advantage, for example by shortening the photoperiod or limiting it to some phenological stages (Niu et al., 2022), or considering the cultivation of more economically profitable cultivars on the market, such as cherry tomatoes (EC, 2022).

4.2 Alternative applications of supplemental LED light for tomato cultivation

Beside yield increase, supplemental LED can be used to foster different objectives and solve some specific issues. For instance, **Exp. 1** demonstrated that the use of supplemental LED light has potential to solve the issue of light limitations determined by safety codes (e.g., structural, fire, seismic) in case of i-RTGs. Furthermore, supplemental LED light can influence other sectors, given its effects on plants metabolism. Indeed, light can be perceived by plants photoreceptors, triggering specific responses depending on light wavelength, intensity, duration and direction (Fankhauser and Chory, 1997; Christie et al., 2015). These responses can be both morphological and physiological, also influencing secondary metabolites production (Ozunis et al., 2015). For instance, the phytochrome, the plant photoreceptor triggered by Red light, has been associated to the capacity to regulate lycopene content in tomatoes (Alba et al., 2000).

4.2.1 Amelioration of nutraceutical traits

The improvement of tomato nutraceutical characteristics is an interesting aspect to achieve with supplemental LED light application. In the present thesis, the nutraceutical properties of tomato seemed to be influenced in different ways according to context and type of metabolite analyzed. In **Exp. 1**, the content of lycopene and β-carotene did not seem to be influenced by LED light, while antioxidant capacity and total phenol content resulted significantly higher in red tomatoes grown under supplemental LED light during the spring period. The same absence of significant differences for lycopene and β-carotene content was also observed during **Exp. 4**. While Palmitessa et al., (2021b) also observed an increased content of antioxidant capacity, the absence of significant increase of lycopene seems to be in contrast with what reported in other researches (Alba et al., 2000, Nájera et al., 2018; Dannehl et al., 2021). However, the absence of differences in carotenoids content in the present research may be related to a not direct or limited exposition to LED light.

4.2.2 Maintaining of quality and food losses reduction during post-harvest

In **Exp. 2**, although lycopene and β-carotene did not show a significant increase during cultivation, they presented a significant higher value in case of RB treated plants as compared to CK after one week of storage at 13°C. This difference can be attributable to a different decay time of carotenoids instead to the biosynthesis of new molecules, as lycopene in red tomatoes stored at 13°C tends to undergo a decay process (Farneti et al., 2012). Fruit hardness was also significantly higher in RB and FR treated tomatoes, which may be determined by an increased cuticular thickness leading to a reduced transpiration rate (Cozmuta et al., 2016). Maintaining of fruit hardness could be an effect of great interest, considering that the majority of food losses are related to post-harvest handling (Rosegrant et al., 2018).

4.2.3 Application for seedling production sector

Excessive elongation of tomato seedling can negatively influence fruiting and assimilates partitioning (Jeong et al., 2020). Moreover, compact plants can better tolerate transportation and transplanting in the cultivation site (Kubota et al., 2004). In some cases, seedlings with long hypocotyls can be desirable, as in the case of grafting operations to guarantee good resistance to different stresses, like temperature, drought, nutrients loss, and pests (Singh et al., 2017). Long hypocotyls can reduce scion cut exposure to soil and disease, also facilitating rootstock grafting (Chian and Kubota, 2010). **Exp. 3** showed that tailored LED light can help to achieve the production of green and compact plants in case of RB and FR treatments, or long hypocotyls in case of EOD treatment, opening to interesting prospective in seedling production sector.

5 CONCLUSIONS AND ANSWERS TO RESEARCH QUESTIONS

From the results, it is possible to conclude that the application of supplemental LED light on greenhouse-grown tomato, with a specific focus on the Mediterranean countries, has potential to foster diverse applications. In particular, it can increase production in case of the limited solar radiation in i-RTGs, maintain quality and reduce losses during post-harvest, and enable the production of tailored and quality seedlings. In particular, the research answered the following research questions:

- **RQ1: What is the current state of tomato cultivation with additional LED light and what aspects still need to be explored? (Chapter 1 and 2).**

Tomato cultivation with supplemental LED light is a practice gaining attention by research, especially in high latitude countries were it can result in a more cost-effective application. However, research is also expanding in lower latitude countries, demonstrating its capability to increase yield capacity. Some areas of application still need to be deepen, also in Mediterranean countries, including tomato quality, post-harvest storage, seedling production, and rooftop greenhouse cultivation.

- **RQ2: What is the potential of supplemental LED light to reduce structural shadings and limited transmission of solar radiation in case of tomato cultivation in Integrated Rooftop Greenhouses (i-RTGs)? (Chapter 3).**

The application of supplemental LED light in an i-RTG has the potential to overcome light limitations due to structural shadings and low transmissivity of fireproof covering materials independently of spectrum quality. In particular, LED treated plants can achieve a yield increase by 17% as compared to the control grown under natural light only (CK), which showed 9.3% lighter and 7.2% fewer fruits.

- **RQ3: Could supplemental LED light during cultivation affect post-harvest quality of tomato? (Chapter 4).**

Pre-harvest application of LED supplemental lighting can positively affect the post-harvest quality of tomatoes after one week of storage at 13°C. Particularly, RB and FR increased fruit firmness compared to CK. Furthermore, RB fruit maintained a higher content of lycopene and β-carotene after one week of storage as compared to CK.

- **RQ4: Beside fruit production, could the application of supplemental LED light interest other commercial areas such as tomato seedlings production? (Chapter 5).**

Supplemental LED light can be an interesting technology to produce tailored and quality seedlings. Indeed, it showed to affect growth indexes and morpho-physiological response of tomato seedlings depending on lighting treatment. In particular, RB and FR treatments might be interesting to produce compact and green plants, which can tolerate transportation and transplanting to cultivation site. Among treatments supplied with artificial lighting, EOD plants presented longer hypocotyls, still maintaining high chlorophyll content, opening to interesting prospective for tomato grafting. On the other hand, CK plants presented the longest hypocotyls, highest leaf area and lowest chlorophyll content, also showing high Specific Leaf Area (SLA) and Leaf Area Ratio (LAR).

- **RQ5: Could the use of additional LED light influence some management aspects of tomato cultivation such as defoliation, whit consequences on qualitative and quantitative aspects of production? (Chapter 6).**

The application or not of defoliation seems to have not significant effects on tomato plants yield and other vegetative parameters, independently of combination with supplemental LED light (RB) or natural light alone (CK). However, plants subjected to leaf removal showed a significantly decreased content of soluble solids, also presenting a higher transpiration. The application of RB treatment showed a significant capacity to increase tomato total yield (+118%) as compared to CK during the wintertime.

6 FUTURE PERSPECTIVES

Despite the positive results obtained, some aspects of the application of additional LED light in Southern Europe countries still need to be deepened and improved. In particular, given the current increase of electricity cost, future research should focus on more economically valuable methods to manage supplemental lighting, such as the application of shorter photoperiods or lower intensities, or techniques that can provide energy savings such as the pulsed light. Further investigation should also concern post-harvest quality evaluation, considering longer storage periods and evaluations of tomatoes at earlier stages of development. Moreover, the EOD treatment, which showed a greater elongation of the hypocotyls in tomato seedlings, should be tested on grafting genotypes to assess the feasibility for this specific sector, and favor the production of easier manageable and higher quality rootstocks. Finally, the extension of tomato seedlings evaluation until fruiting stage should also be considered to better understand the effects that additional light at the beginning of plant development may have on the distribution of assimilates and yield.

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“Cercate di fare anche voi la vostra parte

per questo nostro difficile Paese.”

P. Angela

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