Annals of Agricultural Sciences xxx (xxxx) xxx–xxx

HOSTED BY

Contents lists available at ScienceDirect

Annals of Agricultural Sciences

journal homepage: www.elsevier.com/locate/aoas

Phenolic content and antioxidant activity of einkorn and emmer sprouts and wheatgrass obtained under different radiation wavelengths

Paolo Benincasa, Giacomo Tosti, Michela Farneselli, Stefano Maranghi, Elisabetta Bravi, Ombretta Marconi, Beatrice Falcinelli*, Marcello Guiducci

Dipartimento di Scienze Agrarie, Alimentari ed Ambientali, Università di Perugia, Borgo XX Giugno, 74-06121 Perugia, Italy

ARTICLE INFO

Keywords:
Elicitation
Light
Radiation use efficiency
Phytochemical
Antioxidant
Germination

ABSTRACT

Sprouted seeds represent intriguing ready-to-eat micro-scale vegetables for the healthy food market, since they are tasty and rich in bioactive compounds. However, sprouts have been recently proposed as a source for the extraction and purification of several phytochemicals to be used in food supplementation or pharmaceutics. Recently, there has been an industrialization of sprout production, carried out indoor, often with use of artificial light, which have implications on biomass yield and composition, and on energetic and economic costs. This work investigates the effects of different radiation wavelengths from light emitting diodes (LED) on free and bound phenolics and antioxidant activity of sprouts and wheatgrass of einkorn (*Triticum monococcum* L. ssp. *monococcum*) and emmer ([(*Triticum turgidum* L. spp. *dicoccum*, (Schrank ex Schübler) Thell.)]). After 3 days of grain incubation in the dark, three light treatments were applied, labelled as BLUE (447 and 470 nm), RED (627 and 655 nm), and SUN (447, 470, 505, 530, 590, 627, 655 nm), for a same total photon flux density (PFD) of 200 μmol m^{-2} s^{-1}. Sprouts were harvested at 5 days after sowing (DAS) and wheatgrass at 9 DAS. The effect of light was generally not significant for sprouts, much greater and species-specific for wheatgrass: BLUE in einkorn and RED in emmer generally increased free and total content of polyphenol (PC), tannins (TC), flavonoid (FC) and phenolic acids (PAs). The antioxidant activity was increased by BLUE in einkorn and decreased by RED in both species. BLUE and RED resulted energy saving compared to SUN.

1. Introduction

Cereal sprouts (i.e., young seedlings) and grass (i.e., seedlings a few days older) are currently recognized by scientific literature and consumers as an important source of health-promoting compounds (e.g., phenolic compounds, carotenoids, etc.) (Benincasa et al., 2019; Gan et al., 2019).

Besides the use as ready-to-eat vegetables, in recent years, sprouts have been proposed and studied as a source for the extraction and purification of several phytochemicals to be used in food supplementation or pharmaceutics (Dias et al., 2012; Falcinelli et al., 2018). Einkorn (*T. monococcum* L. ssp. *monococcum*) and emmer [(*T. turgidum* L. spp. *dicoccum*, (Schrank ex Schübler) Thell.)] have been found to have a high content of polyphenols and PAs (Benincasa et al., 2015). Of these, the free/soluble forms (i.e., aglycones, free phenolic acids, glycosides, esters) are known to inhibit LDL cholesterol oxidation, while the bound/insoluble forms (phenolics covalently conjugated through ester bonds to cell wall components like cellulose, pectin and polysaccharides) have chemo-preventive activity against colon cancer (Acosta-Estrada et al., 2014). Environmental stresses are known to affect the phenolic content and the ratio between free and bound forms (Mert-Türk, 2002). As an example, two recent researches (Falcinelli et al., 2017a; Stagnari et al., 2017) demonstrated that a moderate salt stress slightly reduced the growth of einkorn and emmer sprouts and wheatgrass while it markedly enhanced their polyphenol and PAs content, especially the free forms. For this reason the authors proposed the application of moderate salinity as an elicitor to increase the phenolic yield and related antioxidant activity.

The radiation intensity and spectrum may represent another elicitation factor (Bian et al., 2015). Photoresponse is a wavelength-dependent reaction (van Ieperen, 2012). Blue (400–500 nm) and red (600–700 nm) are the major wavelengths perceived by plant photoreceptors (i.e., phototropins or cryptochromes for blue light, and phytochromes for red light) and those that mainly affect photosynthesis and primary metabolisms. Recently, it has been reported that exposure to blue or red lights affects also the production of secondary metabolites, even if the effect seems to vary among species and phytochemical classes (Bian et al., 2015; Demotes-Mainard et al., 2016; Huché-Thélier

* Corresponding author.
E-mail address: beatricefalcinelli90@gmail.com (B. Falcinelli).

https://doi.org/10.1016/j.aoas.2020.02.001
Received 22 October 2019; Received in revised form 12 February 2020; Accepted 14 February 2020

et al., 2016; Samuolienė et al., 2017). Since both homemade and specialized production of sprouts and wheatgrass is carried out indoor, this implies the use of artificial light. Thus, the light spectrum and intensity can be modulated according to producer's requirements (Taulavuori et al., 2017), with the multiple purpose of reducing energy consumption and obtaining high biomass and phytochemical yield. Compared to other lamps (incandescent or HPS lamps), LEDs represent a recent technologies with a longer lifespan, narrower light spectrum, lower heat emission and power consumption. LED lamps may be assembled to produce light in specific wavelengths of the spectrum (Taulavuori et al., 2017). The effects of red and blue light on phenolics in sprouts have been studied for several species (Shimizu, 2016), including grains (Urbonavičiūtė et al., 2009a, b; Samuolienė et al., 2011), but never for einkorn and emmer.

Thus, the aim of this work was to study the effects of red and blue light on free and bound phenolic compounds and on the antioxidant activities of einkorn and emmer sprouts and wheatgrass.

2. Materials and methods

2.1. Plant material and sprouting

Grains of einkorn (*T. monococcum* L. ssp. *monococcum*, cv. Monlis, TMoM) and emmer [*T. turgidum* L. spp. *dicoccum,* (Schrank ex Schübler) Thell., cv. Zefiro, TDiZ] were incubated on filter paper laid over sterile cotton contained in plastic trays (10 g of seeds per tray) and wetted with distilled water (150 mL) to guarantee constant water availability throughout the incubation period, while preventing anoxia (Falcinelli et al., 2017a). The trays were placed in a growth chamber in dark conditions until three DAS, when most of seeds were germinated. Three different light treatments were then applied, labelled as BLUE (447 and 470 nm), RED (627 and 655 nm), and SUN (447, 470, 505, 530, 590, 627, 655 nm), all treatments having the same total PFD of 200 μmol m^{-2} s^{-1} (Table 1) and light/dark photoperiod of 10/14 h. Treatments were laid down according to a completely randomized design with three replicates (trays). Light treatments were built using the same LED-lamps (DSA3-lamps) used by Tosti et al. (2017). The combination of wavelengths, incident photon flux density (PDF), radiation emitted, and energy consumed of the three light treatments are reported in Table 1. The temperature and the relative humidity in the growth chamber were kept at 20 ± 1 °C and 70 ± 5%, respectively.

Sprouts were harvested at 5 DAS collecting the whole plant material (i.e., shoot, root and the seed coat), while wheatgrass was harvested at 9 DAS, collecting only the shoots. Sampled material was stored at −20 °C until analytical determinations, performed in triplicate. Fresh and oven dry weights and the lengths of shoots and roots were measured on a subsample of 10 individuals per replicate.

2.2. Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH), gallic (GA), α-resorcylic, tyrosol, gentisic (GeA), *p*-hydroybenzoic (*p*-HA), 2,6-dihydroxybenzoic, *m*-hydroxybenzoic (*m*-HA), vanillic (VA), salicylic (SaA), syringic (SyA), homovanillic (HoA), *p*-coumaric (*p*-CA), m-coumaric (*m*-CA), *o*-coumaric (o-CA), ferulic (FA), sinapic (SiA), caffeic (CaA), and chlorogenic acid (ChA) were purchased from Sigma Aldrich (St. Louis, MO, USA). All other chemicals used were of an analytical grade.

2.3. Extraction of free and bound phenolic fractions

The extraction of free and bound phenolic fractions was achieved following the method of Krygier et al. (1982) with slight modifications. Frozen sprouts or wheatgrass (1 g) were mixed with 20 mL of methanol:acetone:water (7:7:6) and homogenized on ice using an Ultraturrax for three times, alternating 30 s homogenization and 30 s pause to prevent the material from heating. Samples were then centrifuged at 5000 rpm for 10 min and the supernatant (free fraction) was recovered. The remaining solid residue was mixed with 5 mL of NaOH (5 M) for 1 h and then HCl (5 M) was added until pH = 2. Samples of bound fraction were mixed with 10 mL of ethyl acetate, vortexed and centrifuged at 3000 rpm for 10 min and the supernatant was then recovered (bound fraction). This extraction was performed three times and the supernatants were pooled, evaporated to dryness using a rotary evaporator, and dissolved in 10 mL of methanol 50%. Aliquots of phenolic extracts for the free and bound fractions were used for determination of PC, TC, FC and antioxidant activities.

2.4. Measurement of phenolic compounds

The PC was performed following the method of Singleton and Rossi (1965) with phosphomolybdic – phosphotungstic acid reagent (Folin-Ciocalteu reagent). An aliquot (0.4 mL) of phenolic extract was mixed with 2 mL of Folin-Ciocalteu reagent (1:10) and 1.6 mL of 7.5% sodium carbonate (Na_2CO_3). After 2 h the absorbance was read at 765 nm.

The FC and TC were measured following the same procedure used by Falcinelli et al. (2017b, 2017c) An aliquot (1 mL) of phenolic extract from free and bound fraction was used for each test. FC was calculated by subtracting the non-flavonoid content from PC. Gallic acid was used as a standard, and results were expressed as mg gallic acid equivalent (GAE) g^{-1} dry weight (DW) of sample. The sum of free and bound fractions (total) was calculated for each phenolic class.

2.5. PAs

2.5.1. PAs extraction (free and bound)

The extraction of free and bound phenolic fractions was achieved following the method of Stagnari et al. (2017) Frozen sprouts or wheatgrass (1 g) were homogenized with Ultra-Turrax adding 5 mL of CH3OH/water/acetic acid (70/29.5/0.5) and sonicated for 40 min at room temperature. The mixture was centrifuged at 4000 *g* for 10 min and the supernatant was recovered. The entire extraction was repeated twice. The final extract was evaporated to dryness in rotary evaporator,

Table 1
Incident photon flux density (PFD), radiation emitted (W m^{-2}), and electricity consumed (W m^{-2}) for any of the radiation wavelengths (and nominal colours) in SUN, BLUE and RED light treatments.

Radiation wavelengths	Colour	Incident PFD (μmol m^{-2} s^{-1})			Radiation emitted (W m^{-2})			Electricity consumed (W m^{-2})		
λ (nm)		SUN	BLUE	RED	SUN	BLUE	RED	SUN	BLUE	RED
447.5	Royal blue	25	100	0	6.70	26.80	0	36.41	145.65	0
470.0	Blue	26	100	0	6.63	25.50	0	51.39	196.67	0
505.0	Cyan	28	0	0	6.65	0	0	100.76	0	0
530.0	Green	29	0	0	6.56	0	0	128.63	0	0
590.0	Amber	31	0	0	6.30	0	0	63.64	0	0
627.0	Red	31	0	100	5.93	0	19.12	72.32	0	233.17
655.0	Deep red	31	0	100	5.68	0	18.31	31.38	0	101.16
Total		200	200	200	44.45	52.30	37.43	484.53	342.32	334.33

the residue was re-dissolved in phase A (0.1 M citric acid and 0.2 M Na_2HPO_4; 85:15; v:v), and filtered through 0.45 µm nylon filters, before injection into HPLC system. The solid residue, after the extraction of free PAs, was hydrolyzed with 10 mL of 4 M NaOH by sonication for 40 min at room temperature and treatment at room temperature overnight. The hydrolyzed mixture was adjusted at pH 2 with HCl 6 M and extracted three times with 20 mL of ethyl acetate. The supernatant was recovered and evaporated to dryness, re-dissolved in phase A and injected into HPLC system.

2.5.2. HPLC analysis of PAs

The quantitative analysis of PAs was carried out using external standard calibration, the linearity of calibration plots were between 0.3 and 6 µg/mL and the square of the correlation coefficients were $R^2 > 0.9965$. The following equipment was utilized for the HPLC analysis: a quaternary Azura P 6.1 L pump (Knauer, Berlin, Germany), a Knauer 3950 autosampler with a 10-µL loop, and an Azura MWD 2.1 L UV–VIS detector. The system was managed by Clarity Chromatography Software for Windows (DataApex, Prague, Czech Republic).

The chromatographic separation was achieved at room temperature with a SunShell C18 column (ChromaNik Technologies Inc. 50 mm × 2.1 mm ID). The three wavelengths for the determination of PAs were 254, 278, and 324 nm. Mobile phase A was 0.1 M citric acid and 0.2 M Na_2HPO_4 (85:15; v:v), and mobile phase B was phase A/CH_3-OH/CH_3CN, 30:20:50 *v*/v/v. The mobile phases were adjusted to pH 3.44 with 85% orthophosphoric acid and were filtered with a 0.22 µm membrane filter (Millipore, Bedford, MA, for aqueous solvents; MSI, MA, for organic solvents). The solvent gradient and flow rate during analysis are reported in Table 2. It represents the same frame of the program in the pump.

2.6. Antioxidant activity

Antioxidant activity was measured by DPPH and ferric reducing antioxidant power (FRAP) tests following the same procedure used by Falcinelli et al. (2017b). An aliquot (150 µL) of phenolic extract from free and bound fraction was used for each test. The results of all tests were expressed in µmol Trolox equivalents (TE) g^{-1} DW of sample.

2.7. Statistical analysis

All data were analyzed by two-way ANOVA and the effect of genotype (*G*), light (*L*), and their interaction (*G* × *L*) were tested. Average values of triplicate determinations ± standard error are depicted. The R statistical environment was used to perform the analysis (R Core Team, 2014). Differences between any couple of treatments were tested by the least significant difference (LSD) of the interaction.

3. Results

3.1. Growth parameters

Growth parameters (i.e., individual fresh weight and dry matter content, and shoot and root lengths) of einkorn and emmer sprouts and wheatgrass are reported in Table 3. In sprouts, the effect of *G* was always significant, with the highest values in TDiZ, while the light treatment *L* significantly affected only the root length, with higher values in RED. In wheatgrass, *L* significantly affected all growth indexes. In particular, compared to SUN, RED increased the fresh weight and the shoot length in both TMoM and TDiZ, but decreased the dry matter content.

Table 2
Solvent gradient and flow rate during HPLC analysis.

Step	Time (min)	A%	Flow (ml min^{-1})
Initial	0.0	90	0.4
1	2.0	100	0.4
2	8.0	70	0.4
3	10.0	50	0.4
4	12.0	20	0.4
5	12.5	90	0.4

Table 3
Individual fresh weight (g), dry matter content (%), and shoot and root lengths (mm) of einkorn (TMoM) and emmer (TDiZ) sprouts and wheatgrass under three light treatments differing for radiation wavelengths: BLUE (447 and 470 nm), RED (627 and 655 nm), and SUN (447, 470, 505, 530, 590, 627, 655 nm). The three treatments had a same overall photon flux density (PFD) of 200 µmol m^{-2} s^{-1}. Standard errors in brackets. G: Genotype; L: light; LSD: Least significance difference of interaction (G × L) for $P = .05$; n.c.: not collected; n.s.: not significant.

Samples		Fresh weight (g)	Dry matter content (%)	Length (mm)	
				Shoot	Root
Sprouts					
TMoM	SUN	0.12 (0.007)	19.0 (0.71)	39 (2.4)	30 (1.5)
	BLUE	0.12 (0.002)	21.3 (1.04)	35 (1.3)	30 (1.0)
	RED	0.15 (0.003)	19.1 (0.18)	47 (2.5)	41 (3.1)
TDiZ	SUN	0.34 (0.005)	22.6 (1.00)	72 (0.8)	84 (3.0)
	BLUE	0.33 (0.016)	22.7 (0.18)	70 (2.1)	83 (0.4)
	RED	0.36 (0.033)	22.9 (1.15)	69 (2.9)	89 (0.8)
Significance	G	**	**	**	**
	L	n.s.	n.s.	n.s.	**
	G × L	n.s.	n.s.	*	n.s.
	LSD	0.06	3.52	9.27	8.31
Wheatgrass					
TMoM	SUN	0.12 (0.004)	13.3 (0.29)	125 (4.9)	n.c.
	BLUE	0.14 (0.016)	12.9 (0.28)	90 (3.4)	n.c.
	RED	0.17 (0.011)	12.0 (0.01)	149 (3.8)	n.c.
TDiZ	SUN	0.16 (0.010)	13.0 (0.09)	174 (4.7)	n.c.
	BLUE	0.14 (0.007)	12.6 (0.12)	142 (5.7)	n.c.
	RED	0.17 (0.009)	11.8 (0.12)	190 (7.3)	n.c.
Significance	G	n.s.	n.s.	**	–
	L	*	**	**	–
	G × L	n.s.	n.s.	n.s.	–
	LSD	0.04	0.79	22.17	–

* Indicates significance at $P < .05$.
** Indicates significance at $P < .01$.

3.2. PC, TC and FC

The PC, TC and FC changed with growth stages and light treatments (Table 4). In sprouts of both TMoM and TDiZ, the free fraction of PC, TC and FC was approximately half of the total, while in wheatgrass, it was the greatest part (generally over 90% of the total) due to an increase of the free fractions and a decrease of the bound ones, especially in TMoM. Overall, the total-PC, -TC and -FC passing from sprouts to wheatgrass increased in TDiZ and did not change markedly in TMoM.

In sprouts, PC, TC and FC were significantly affected only by *G* with higher values in TMoM than in TDiZ for each phenolic class and fraction, except for free-TC. In wheatgrass, the bound fractions of PC and TC did not differ significantly, while significant effects of *L* and *G x L* were recorded for free and total PC, TC and FC. Compared to SUN, BLUE increased significantly the free- and total-PC, -TC, and -FC in TMoM, while it was mainly RED, and sometimes BLUE, that increased these compounds in TDiZ.

3.3. PAs

The PAs content changed with the growth stage and some PAs were detected in sprouts (Table 5) but not in wheatgrass (Table 6) or *vice versa*, while some PAs were never detected (gallic, α-resorcylic, tyrosol, 2,6-dihydroybenzoic, *o*-coumaric). Considering the overall PAs content

Table 4
Contents of free, bound and total polyphenols (PC), tannins (TC) and flavonoids (FC) in einkorn (TMoM) and emmer (TDiZ) sprouts and wheatgrass under three light treatments differing for radiation wavelengths: BLUE (447 and 470 nm), RED (627 and 655 nm), and SUN (447, 470, 505, 530, 590, 627, 655 nm). The three treatments had a same overall photon flux density (PFD) of 200 μmol m^{-2} s^{-1}. Standard errors in brackets. DW: dry weight; G: Genotype; GAE: gallic acid equivalents; L: light; LSD: Least significance difference of interaction G × L for P = .05; n.s.: not significant.

Treatments		Phenolics (mg GAE g^{-1} DW)								
		PC			TC			FC		
		Free	Bound	Total	Free	Bound	Total	Free	Bound	Total
Sprouts										
TMoM	SUN	11.4 (0.53)	13.0 (0.12)	24.4 (0.48)	6.0 (0.24)	4.1 (0.49)	10.1 (0.26)	8.06 (0.62)	7.7 (0.33)	15.8 (0.94)
	BLUE	11.7 (0.59)	14.8 (0.51)	26.5 (1.10)	6.2 (0.32)	8.2 (1.37)	14.5 (1.38)	8.2 (0.68)	12.7 (1.44)	20.9 (1.88)
	RED	10.2 (1.49)	12.5 (0.67)	22.7 (1.51)	5.8 (1.29)	5.1 (1.50)	10.9 (1.03)	7.1 (1.2)	9.7 (1.42)	16.7 (0.33)
TDiZ	SUN	8.7 (1.37)	9.4 (1.05)	18.1 (3.38)	4.4 (1.46)	3.6 (0.82)	8.1 (2.28)	6.1 (1.24)	6.2 (0.75)	12.2 (1.91)
	BLUE	8.9 (1.28)	10.4 (1.70)	19.4 (2.53)	4.9 (1.20)	3.8 (1.62)	8.7 (1.01)	6.1 (1.06)	7.1 (1.73)	13.2 (2.16)
	RED	7.9 (0.75)	7.8 (0.17)	15.8 (0.67)	4.3 (0.95)	2.9 (0.09)	7.21 (1.01)	5.5 (0.82)	5.2 (0.18)	10.6 (0.77)
Significance	G	*	**	**	n.s.	*	**	*	**	**
	L	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	G × L	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	LSD	4.6	3.8	7.1	4.4	4.9	5.7	4.1	4.9	6.5
Wheatgrass										
TMoM	SUN	21.0 (0.88)	0.5 (0.05)	21.5 (0.87)	6.1 (0.44)	n.d.	6.1 (0.44)	13.8 (0.64)	0.1 (0.01)	13.8 (0.63)
	BLUE	30.5 (1.03)	1.2 (0.06)	31.7 (1.07)	10.9 (1.46)	n.d.	10.9 (1.46)	23.5 (4.45)	0.5 (0.19)	24.0 (4.60)
	RED	21.6 (1.54)	1.5 (0.95)	23.1 (2.10)	6.3 (0.68)	n.d.	6.3 (0.68)	14.6 (1.02)	1.0 (0.95)	15.7 (1.75)
TDiZ	SUN	19.3 (1.92)	2.1 (0.09)	21.4 (2.01)	11.0 (1.33)	n.d.	11.0 (1.33)	12.7 (1.10)	1.6 (0.05)	14.2 (1.15)
	BLUE	24.1 (1.96)	1.4 (0.24)	25.4 (1.86)	10.1 (1.52)	n.d.	10.1 (1.52)	16.9 (1.38)	0.9 (0.21)	17.7 (1.28)
	RED	24.5 (0.77)	2.0 (0.32)	26.5 (0.49)	16.5 (0.77)	0.68 (0.30)	17.2 (0.57)	17.5 (0.75)	1.4 (0.21)	18.9 (0.55)
Significance	G	n.s.	n.s.	n.s.	**	–	**	n.s.	*	n.s.
	L	**	n.s.	**	n.s.	–	*	*	n.s.	*
	G × L	*	n.s.	*	**	–	**	n.s.	n.s.	n.s.
	LSD	6.2	1.8	6.6	4.8	–	4.7	8.8	1.8	9.3

* Indicates significance at $P < .05$.
** Indicates significance at $P < .01$.

(Σ), Σ of free PAs increased passing from sprouts to wheatgrass, while Σ of bound and total PAs decreased.

In sprouts, bound-PAs represented the greatest part of total-PAs (Table 5). Free-SyA and bound-p-CA, -FA and -SaA were the most represented PAs. The Σ of free, bound and total PAs showed always significant differences for the interaction *G* × L. In both genotypes, both BLUE and RED generally increased significantly Σ free-PAs compared to SUN, while Σ of bound- and total-PAs were increased by RED and decreased by BLUE (except in TDiZ).

In wheatgrass, bound-PAs were generally still higher than free-PAs (Table 6). Free-GeA, -SyA and -FA, and bound-FA were the most represented PAs. The Σfree-PAs was significantly affected only by *L*, while the Σbound- and Σtotal-PAs were significant affected by both *L* and the interaction *G x* L. For each fraction, the highest values were recorded with BLUE for TMoM (especially free-p-CA, -FA and -SaA; bound-p-CA, -FA and -SiA; total-p-CA, -FA) and RED for TDiZ (free-p-CA and -SaA; bound-p-CA, -m-CA and -SiA; total-p-CA, -m-CA and -SaA).

3.4. Antioxidant activity

The antioxidant activity measured by DPPH and FRAP are reported in Table 7. In sprouts, the free fraction showed values comparable to those of the bound fraction for both tests. The interaction *G* × *L* was significant for DPPH and FRAP of the bound fraction and FRAP of the free fraction. In TMoM, both BLUE and RED reduced the antioxidant activity compared to SUN, while in TDiZ the effect was generally not relevant.

In wheatgrass, the antioxidant activity of free phenolics increased, while that of bound phenolics decreased markedly and became risible in both TMoM and TDiZ. Considering the free fraction, the highest values in TMoM were recorded with BLUE, while the lowest in both TMoM and TDiZ were recorded with RED.

4. Discussion

Growth parameters recorded in SUN for sprouts and wheatgrass were in line with those observed by Falcinelli et al. (2017a) in the same cultivars of both einkorn and emmer (Table 3). The lack of a significant effect of light on most growth parameters in sprouts maybe due to the short time of exposition to light (i.e., 2 days). Only sprout root length was significantly increased by RED, which appears surprising, since it has been widely reported that root elongation is generally decreased by red light (Demotes-Mainard et al., 2016). However, root elongation is a very complex mechanism regulated by phytochrome (Correll and Kiss, 2005; Demotes-Mainard et al., 2016) and Salisbury et al. (2007) found that phytochrome A, B and E promoted lateral root production in *Arabidopsis* seedlings. Further experiments may be necessary to confirm and explain this evidence. As far as wheatgrass is concerned, the effects we observed for BLUE and RED treatments are consistent with the literature available for adult plants reviewed by Demotes-Mainard et al. (2016) for blue light and Huché-Thélier et al. (2016) for red light. In particular, the increase of stem elongation with red light is again to be explained as a consequence of phytochrome regulation. On the other hand, blue light is known to reduce cell wall extensibility (Huché-Thélier et al., 2016) and induce a compact appearance of plants (Ouzounis et al., 2015). The effect of both red and blue light treatments depends on plant species and genotype (Demotes-Mainard et al., 2016; Huché-Thélier et al., 2016). Moreover, the photosynthetic activity is reduced, compared to white light, when blue light is alone or when blue wavelengths are missing in the light spectrum (Huché-Thélier et al., 2016) (i.e., our red light treatment, RED). This might explain our results on dry mass.

The increase of PC in einkorn and emmer, passing from sprouts to wheatgrass, is in line with our previous evidence (Benincasa et al., 2015; Falcinelli et al., 2017a; Stagnari et al., 2017) (Table 4). The higher content of PC recorded in this study, compared to the above

Table 5
Contents of free, bound and total phenolic acids (PAs) (μg g^{-1} dry weight, DW) in einkorn (TMoM) and emmer (TDiZ) sprouts under three light treatments differing for radiation wavelengths: BLUE (447 and 470 nm), RED (627 and 655 nm), and SUN (447, 470, 505, 530, 590, 627, 655 nm). The three treatments had a same overall photon flux density (PFD) of 200 μmol m^{-2} s^{-1}. Standard errors in the brackets. *p*-CA: *p*-coumaric acid FA: ferulic acid, G: Genotype; GeA: gentisic acid; *p*-HA: *p*-hydroxybenzoic acid; *m*-HA, *m*-hydroxybenzoic acid; HoA: homovanillic acid; L: light; LSD: Least significance difference of interaction (G × L) for P = .05; n.d.: not detected; n.s.: not significant; SaA: salicylic acid; SiA: sinapic acid; SyA: syringic acid; VA: vanillic acid; Σ: sum of single PAs.

Treatments		PAs (μg g^{-1} DW)										Σ
		Hydroxybenzoic acids						Hydroxycinnamic acids			Other class	
		GeA	*p*-HA	*m*-HA	SaA	SyA	VA	*p*-CA	FA	SiA	HoA	
Free												
TMoM	SUN	49.3 (1.04)	7.3 (0.33)	n.d.	n.d.	500.5 (20.63)	n.d.	n.d.	4.62 (0.34)	n.d.	n.d.	561.7 (19.02)
	BLUE	41.1 (2.03)	15.5 (1.80)	n.d.	n.d.	701.3 (62.63)	n.d.	n.d.	3.0 (0.08)	n.d.	n.d.	761.0 (40.22)
	RED	29.0 (0.34)	5.2 (0.58)	n.d.	n.d.	800.0 (32.68)	n.d.	n.d.	2.62 (0.28)	n.d.	n.d.	836.9 (32.52)
TDiZ	SUN	42.9 (2.96)	3.1 (0.09)	n.d.	n.d.	300.6 (3.58)	n.d.	n.d.	5.40 (0.11)	n.d.	n.d.	352.0 (6.26)
	BLUE	89.8 (6.64)	2.3 (0.24)	n.d.	n.d.	732.4 (19.40)	n.d.	n.d.	5.07 (0.53)	n.d.	n.d.	829.6 (24.42)
	RED	48.3 (7.20)	1.2 (0.14)	n.d.	n.d.	529.4 (20.58)	n.d.	n.d.	2.65 (0.21)	n.d.	n.d.	581.5 (25.35)
Significance												
G		**	**	–	–	**	–	–	**	–	–	**
L		**	**	–	–	**	–	–	**	–	–	**
G × L		**	**	–	–	**	–	–	*	–	–	**
LSD		18.5	3.4	–	–	107.1	–	–	1.3	–	–	115.9
Bound												
TMoM	SUN	n.d.	7.9 (0.31)	246.5 (10.72)	1696.0 (65.72)	121.7 (2.39)	23.4 (0.44)	403.1 (10.53)	469.1 (13.70)	158.7 (16.84)	46.6 (1.30)	3173.0 (98.82)
	BLUE	n.d.	6.0 (0.24)	169.5 (13.14)	1220.8 (69.12)	57.6 (3.50)	13.4 (0.91)	564.7 (34.70)	493.6 (26.47)	113.5 (7.57)	15.7 (1.44)	2655.0 (151.23)
	RED	n.d.	7.5 (0.10)	113.2 (4.78)	1788.1 (82.99)	73.1 (3.82)	17.7 (1.07)	720.7 (13.10)	630.4 (18.23)	155.8 (9.06)	21.6 (1.17)	3528.1 (57.87)
TDiZ	SUN	n.d.	10.1 (0.04)	117.7 (5.45)	951.1 (7.27)	116.3 (3.02)	18.6 (1.07)	754.7 (7.18)	907.9 (52.43)	215.5 (11.26)	60.7 (6.31)	3152.6 (47.78)
	BLUE	n.d.	8.0 (0.91)	140.1 (1.34)	728.6 (53.85)	78.4 (6.22)	16.1 (2.58)	775.5 (49.11)	932.8 (43.08)	160.0 (18.94)	34.2 (4.23)	2873.8 (161.8)
	RED	n.d.	6.9 (0.31)	209.1 (10.17)	1064.8 (29.01)	85.9 (1.49)	9.5 (0.39)	700.3 (12.98)	811.3 (11.90)	224.6 (4.27)	41.1 (1.47)	3152.7 (61.40)
Significance	G	–	**	*	**	**	**	**	**	**	**	n.s.
	L	–	**	*	**	**	**	**	n.s.	**	**	**
	G × L	–	*	**	n.s.	*	**	**	**	n.s.	n.s.	*
	LSD	–	1.8	36.4	247.8	15.7	5.1	112.7	114.5	51.2	11.0	460.6
Total												
TMoM	SUN	49.3 (1.04)	15.2 (0.64)	246.5 (10.72)	1696.0 (65.72)	622.1 (18.80)	23.4 (0.44)	403.1 (10.53)	473.7 (14.04)	158.7 (16.84)	46.6 (1.30)	3734.6 (80.13)
	BLUE	41.1 (2.03)	21.5 (1.58)	169.5 (13.14)	1220.83 (69.12)	758.9 (36.75)	13.4 (0.91)	564.7 (34.70)	496.6 (26.56)	113.5 (7.57)	15.7 (1.44)	3415.9 (128.32)
	RED	29.0 (0.34)	12.7 (0.63)	113.2 (4.78)	1788.1 (82.99)	873.2 (30.94)	17.7 (1.07)	720.7 (13.10)	633.1 (17.95)	155.8 (9.06)	21.6 (1.17)	4365.0 (42.78)
TDiZ	SUN	42.9 (2.96)	13.2 (0.08)	117.7 (5.45)	951.1 (7.27)	416.9 (4.78)	18.6 (1.07)	754.7 (7.18)	913.3 (31.70)	215.5 (11.26)	60.7 (6.31)	3504.6 (45.20)
	BLUE	89.8 (6.64)	10.3 (1.04)	140.1 (1.34)	728.6 (53.85)	810.9 (23.37)	16.1 (2.58)	775.5 (49.11)	937.9 (43.33)	160.0 (18.94)	34.2 (4.23)	3703.4 (178.09)
	RED	48.3 (7.20)	8.1 (0.25)	209.1 (10.17)	1064.8 (29.01)	614.6 (19.13)	9.5 (0.39)	700.3 (12.98)	814.0 (12.06)	224.6 (4.27)	41.1 (1.47)	3734.3 (46.64)
Significance	G	**	**	*	**	**	**	**	**	**	**	*
	L	**	**	*	**	**	**	**	n.s.	**	**	**
	G × L	**	**	**	n.s.	**	**	**	**	n.s.	n.s.	**
	LSD	18.5	3.7	36.4	247.8	105.8	5.12	112.7	115.0	51.2	11.0	434.3

* Indicates significance at $P < .05$.
** Indicates significance at $P < .01$.

Table 6
Contents of free, bound and total phenolic acids (PAs) (μg g^{-1} dry weight, DW) in einkorn (TMoM) and emmer (TDiZ) wheatgrass under three light treatments differing for radiation wavelengths: BLUE (447 and 470 nm), RED (627 and 655 nm), and SUN (447, 470, 505, 530, 590, 627, 655 nm). The three treatments had a same overall photon flux density (PFD) of 200 μmol m^{-2} s^{-1}. Standard errors in the brackets. *p*-CA: *p*-coumaric acid; *m*-CA: *m*-coumaric acid; CaA: caffeic acid; ChA: chlorogenic acid; G: genotype; GeA: gentisic acid; FA: ferulic acid; L:light; SaA: salicylic acid; SiA: sinapic acid; SyA: syringic acid; n.d.: not detected; n.s.: not significant; LSD: Least significance difference of interaction (G × L) for P = .05; Σ: sum of single PAs.

Treatments		PAs (μg g^{-1} DW)									Σ
		Hydroxybenzoic acids			Hydroxycinnamic acids						
		GeA	SaA	SyA	*p*-CA	*m*-CA	CaA	ChA	FA	SiA	
Free											
TMoM	SUN	142.2 (32.68)	44.8 (2.45)	439.3 (146.6)	5.7 (0.79)	n.d.	117.4 (32.45)	56.8 (13.62)	159.6 (12.32)	n.d.	965.8 (137.17)
	BLUE	221.6 (7.03)	73.5 (3.14)	529.4 (95.58)	15.7 (0.30)	n.d.	156.8 (2.00)	63.3 (3.24)	592.1 (32.20)	n.d.	1652.4 (101.62)
	RED	218.7 (7.74)	52.9 (3.80)	907.6 (284.34)	4.1 (0.45)	n.d.	153.4 (18.04)	71.1 (21.00)	147.5 (18.57)	n.d.	1555.3 (258.17)
TDiZ	SUN	93.5 (1.65)	362.7 (29.48)	327.3 (38.39)	11.5 (0.18)	n.d.	135.7 (6.54)	131.9 (19.88)	27.3 (2.80)	n.d.	1089.9 (58.86)
	BLUE	160.5 (24.08)	508.8 (54.59)	467.0 (163.43)	9.6 (1.52)	n.d.	213.0 (65.39)	95.6 (35.26)	64.69(45.09)	n.d.	1519.2 (283.03)
	RED	238.1 (1.96)	1069.8 (41.62)	470.4 (52.67)	12.0 (0.25)	n.d.	263.8 (39.94)	203.6 (27.16)	19.2 (1.16)	n.d.	2276.9 (73.31)
Significance	G	n.s.	**	n.s.	**	–	n.s.	**	**	–	n.s.
	L	**	**	n.s.	**	–	n.s.	n.s.	**	–	**
	G × L	n.s.	**	n.s.	**	–	n.s.	n.s.	**	–	n.s.
	LSD	94.5	106.21	665.6	2.1	–	189.7	96.8	81.5	–	858.1
Bound											
TMoM	SUN	n.d.	148.8 (55.75)	n.d.	148.2 (32.17)	n.d.	n.d.	n.d.	900.3 (237.25)	225.8 (62.47)	1423.1 (266.16)
	BLUE	n.d.	174.4 (2.11)	n.d.	394.2 (16.69)	n.d.	n.d.	n.d.	1830.1 (330.27)	453.1 (24.22)	2851.8 (363.42)
	RED	n.d.	107.3 (0.74)	n.d.	213.0 (22.44)	n.d.	n.d.	n.d.	1159.5 (111.84)	263.8 (23.50)	1743.6 (133.72)
TDiZ	SUN	n.d.	56.1 (9.57)	n.d.	35.6 (8.59)	8.3 (1.53)	n.d.	n.d.	251.1 (30.55)	75.8 (4.63)	426.8 (27.88)
	BLUE	n.d.	171.2 (77.85)	n.d.	119.8 (65.46)	10.3 (2.14)	n.d.	n.d.	1068.4 (226.65)	223.9 (94.59)	1593.5 (288.29)
	RED	n.d.	257.0 (25.95)	n.d.	230.4 (34.79)	23.5 (3.62)	n.d.	n.d.	1289.9 (68.38)	498.2 (37.36)	2298.9 (156.98)
Significance	G	–	*	–	**	**	–	–	*	n.s.	*
	L	–	**	–	**	**	–	–	**	n.s.	**
	G × L	–	n.s.	–	**	**	–	–	n.s.	n.s.	*
	LSD	–	222.8	–	143.6	7.6	–	–	788.7	297.3	1226.0
Total											
TMoM	SUN	142.2 (32.68)	193.6 (57.22)	439.3 (146.6)	153.9 (22.23)	n.d.	117.4 (32.45)	56.8 (13.62)	1059.8 (229.48)	225.8 (62.47)	2388.8 (144.78)
	BLUE	221.6 (7.03)	247.9 (2.88)	529.4 (95.58)	409.8 (10.74)	n.d.	156.8 (2.00)	63.3 (3.24)	2422.2 (305.63)	453.1 (24.22)	4504.2 (290.96)
	RED	218.7 (7.74)	160.2 (3.06)	907.6 (284.34)	217.1 (22.7)	n.d.	153.4 (18.04)	71.1 (21.00)	1306.9 (130.07)	263.8 (23.50)	3298.9 (215.28)
TDiZ	SUN	93.5 (1.65)	418.9 (35.02)	327.3 (38.39)	47.1 (5.01)	8.3 (1.53)	135.7 (6.54)	131.9 (19.88)	278.4 (16.51)	75.8 (4.63)	1516.7 (70.96)
	BLUE	160.5 (24.08)	680.0 (99.36)	467.0 (163.43)	129.4 (66.32)	10.3 (2.14)	213.0 (65.39)	95.6 (35.26)	1133.1 (145.37)	223.9 (94.59)	3112.7 (508.72)
	RED	238.1 (1.96)	1326.8 (64.69)	470.4 (52.67)	242.4 (34.70)	23.5 (3.62)	263.8 (39.94)	203.6 (27.16)	1309.1 (67.23)	498.2 (37.36)	4575.8 (86.11)
Significance	G	n.s.	**	n.s.	**	**	n.s.	*	**	n.s.	n.s.
	L	**	**	n.s.	**	**	n.s.	n.s.	**	n.s.	**
	G × L	n.s.	**	n.s.	**	**	n.s.	n.s.	*	n.s.	**
	LSD	94.5	297.5	665.6	144.9	7.6	189.7	96.8	766.5	297.3	1478.6

* Indicates significance at $P < .05$.
** Indicates significance at $P < .01$.

Table 7
Antioxidant activity measured by DPPH and FRAP tests (μmol Trolox equivalents g^{-1} DW) in einkorn (TMoM) and emmer (TDiZ) sprouts and wheatgrass under three light treatments differing for radiation wavelengths: BLUE (447 and 470 nm), RED (627 and 655 nm), and SUN (447, 470, 505, 530, 590, 627, 655 nm). The three treatments had a same overall photon flux density (PFD) of 200 μmol m^{-2} s^{-1}. Standard errors in the brackets. G: Genotype; L: light; LSD: Least significance difference of interaction G × L for P = .05; n.d.: not detected; n.s.: not significant.

Treatments		DPPH		FRAP	
		Free	Bound	Free	Bound
Sprouts					
TMoM	SUN	5.7 (0.87)	17.8 (0.17)	60.2 (5.77)	69.0 (1.88)
	BLUE	1.1 (0.26)	11.6 (1.37)	21.3 (2.90)	47.5 (4.0)
	RED	5.4 (0.57)	10.9 (0.96)	16.0 (1.06)	36.3 (4.92)
TDiZ	SUN	10.5 (2.0)	10.1 (0.76)	27.4 (3.77)	38.8 (4.29)
	BLUE	7.3 (0.13)	11.4 (0.07)	29.9 (4.54)	37.61 (5.37)
	RED	9.1 (0.42)	10.3 (0.89)	26.7 (1.26)	32.9 (0.30)
Significance	G	**	**	n.s.	**
	L	**	**	**	**
	G × L	n.s.	**	**	*
	LSD	3.8	3.6	15.7	16.8
Wheatgrass					
TMoM	SUN	32.1 (1.69)	n.d.	74.7 (3.94)	3.8 (0.57)
	BLUE	43.4 (1.63)	n.d.	110.0 (8.78)	4.6 (0.75)
	RED	23.3 (1.59)	n.d.	59.2 (3.08)	3.5 (0.16)
TDiZ	SUN	27.8 (4.15)	n.d.	64.8 (6.88)	4.0 (0.44)
	BLUE	23.1 (4.99)	n.d.	84.1 (13.31)	3.3 (0.18)
	RED	15.6 (1.11)	n.d.	41.5 (4.58)	4.6 (0.81)
Significance	G	**	n.s.	*	n.s.
	L	**	n.s.	**	n.s.
	G × L	*	n.s.	n.s.	n.s.
	LSD	12.4	–	32.9	2.4

* Indicates significance at $P < .05$.
** Indicates significance at $P < .01$.

mentioned ones is due to the different methods used for phenolic extraction (i.e., extraction solvent: methanol:acetone:water) and phenolic quantification, which allowed to include flavonoids and tannins and the bound fraction of polyphenols. Flavonoids are generally pigments (i.e., flavanols, flavanones, anthocyanins, etc.) with many health effects (Xiao et al., 2011), while tannins, known only for the astringent taste and the anti-nutritive properties, were recently reported to have antiseptic, anti-diarrhea and anti-inflammatory properties, and antioxidant properties related to the protection against stomach and duodenal tumors (Khanbabaee and Van Ree, 2001). The importance of including the bound phenolic fraction, generally neglected by most literature, was discussed by Falcinelli et al., 2017c and it is of relevance that bound phenolics in sprouts represented half of the total. The increase of free phenolics passing from sprouts to wheatgrass, might be due to either a release from the bound ones or an *ex novo* synthesis (Ti et al., 2014).

Studies on the effect of radiation wavelengths on polyphenols, flavonoids and tannins are limited for sprouted grains (Urbonavičiūtė et al., 2009a, b; Samuolienė et al., 2011) and the use of different light wavelengths, intensities and exposition times hinders accurate comparisons. The lack of the effect of light treatments on sprouts might be due to the short time of exposition, i.e., just two days. Similarly, a short time of exposition to light treatments resulted in risible effects in buckwheat sprouts (Lee et al., 2014; Thwe et al., 2014).

The general positive effect of BLUE on the production of free phenolics in wheatgrass is in line with available literature. Blue light represents a high energy radiation and plant tissues react by increasing the pool of protective pigments, like flavonoids (Samuolienė et al., 2017; Iwai et al., 2010) whose synthesis is linked to cryptochromes and phototropins (Thwe et al., 2014; Liu et al., 2016; Bantis et al., 2018; Holopainen et al., 2018). Moreover, in lettuce, the activity of phenylalanine ammonia-lyase (PAL), the key enzyme in the phenylpropanoid pathway (Heo et al., 2012), and PAL gene expression (Son and Oh, 2013) were found to be stimulated by blue light. Althought the knowledge on LED light effects on sprouts is limited, there are other key enzymes of phenylpropanoid pathway, which are modulated by blue light like the chalcone isomerase, the flavones synthase, and the anthocyanidin synthase, involved in naringenin, quercetin and cyanidin (i.e., flavonoids) production, respectively (Alrifai et al., 2019). As far as TC are concerned, the effect of blue or red light was not studied yet for sprouts, however, high energy radiation in adult plants (i.e., UV-B) has been found to increase the TC (Rozema et al., 1997), supporting our results in TMoM under BLUE. The different effect of RED in the two genotypes (i.e., positive for emmer and null for einkorn) does not surprise, since Samuolienė et al. (2017) reported that red light could increase, decrease or have no effect on polyphenol content of seedlings, depending on the species.

Our results on PAs are in line with previous evidences of Benincasa et al. (2015) and Stagnari et al. (2017) in einkorn and emmer sprouts and wheatgrass (Table 5–6). In detail: i) bound PAs were higher than free PAs; ii) free PAs increased while bound ones decreased passing from sprouts to wheatgrass; iii) PA forms detected in sprouts were sometimes different from those detected in wheatgrass. As for total PC, the different analytical method used here might explain the higher number of PAs and their overall content compared to our previous studies. Besides the well known PAs detected in those studies (i.e., CA; SyA; p-HA; p-CA; SaA and VA), we found here GeA, m-HA, HoA, FA, SiA, m-CA and ChA. The health benefits of p-CA; FA, GeA, SyA and SaA, the most represented PAs compounds, are well documented (Paterson and Lawrence, 2001; Dykes and Rooney, 2007).

The effect of blue and red light on PAs was not studied by authors who investigated the effect on polyphenols in sprouted grains (Urbonavičiūtė et al., 2009a, b; Samuolienė et al., 2011). Alrifai et al. (2019) reported that blue light had a modulatory effect on cinnamate-4-hydroxlase, which is the enzyme involved in *p*-coumaric acid production from which other hydroxycinnamic acids derive. Samuolienė et al. (2017) reported that the synthesis of PAs can be induced by several wavelengths, although blue light is closely related to their metabolic pathways, and Taulavuori et al. (2017, 2018) reported that the induction of PAs synthesis in plants by blue light is species-specific. Our results on wheatgrass of TMoM confirm the stimulatory effect of blue light, while the results on sprouts of both species and on wheatgrass of TDiZ seem to involve an effect of RED in PAs production. In particular, SaA might be considered as an indicator of plant stress since it is involved in the induction of defense related genes and it has been shown to improve plant tolerance to major abiotic stresses (i.e., metal, salinity, osmotic, drought, and heat stress), including very high radiation energy (i.e., UV-B) (Khan et al., 2015). Results on SaA would confirm that BLUE and RED light may represent a stressing condition and elicit the increase of plant antioxidant pool.

The increase of Σfree-PAs and of Σbound-PAs with BLUE and RED light in wheatgrass is of relevance, considering the fate and health effect of these two forms. Free PAs are rapidly absorbed in the stomach and small intestine, while bound forms need to be released in the gastrointestinal tract by microorganisms, enzymes and even glucose transporters, before being absorbed and exerting their health benefits (Acosta-Estrada et al., 2014).

The increase of antioxidant activity in the free fraction and its decrease in the bound one observed passing from sprouts to wheatgrass would appear associated with the trend of phenolic contents, but no significant correlation was observed, thus other molecules besides polyphenols were likely involved in antioxidant activities (Table 7). With respect to the effect of light treatments, the other authors who investigated on the antioxidant activity of sprouted grains used only one test, the DPPH (Urbonavičiūtė et al., 2009a, b; Samuolienė et al., 2011). In most cases, they reported little effects, generally a reduction of antioxidant activity with red light, but any detailed comparison seems not appropriate because of the different wavelengths and times

of exposition. Our results, based on DPPH and FRAP, seem to suggest that two days of exposition to light (sprout stage) are too few to get any relevant and clear effect, while a week of exposition (wheatgrass stage) may have an effect, which is an increase of antioxidant activity with blue light and a decrease with red light.

The effects of the three different light treatments on phenolic content and antioxidant activity deserve to be discussed also in view of the energy consumption of the LED lamps. From data of radiation emission and electricity consumption reported in Table 1, it comes out that the lamp efficiency (Watt/W) was 66% higher for BLUE and 22% higher for RED as compared to SUN. Thus, using monochromatic light, and in particular the blue wavelengths, not only increased the phenolic content of sprouted grains, but also reduced the energy consumption, ultimately improving the "efficiency of energy conversion into phenolics".

5. Conclusions

Our results indicate that the radiation wavelength affected the phenolic content of sprouted einkorn and emmer, especially at the wheatgrass stage. The effect of light was species-specific: blue light in einkorn and red light in emmer generally increased total PC, TC, FC and PAs as compared to the multi-wave control. The antioxidant activity appeared not to depend only on phenolic compounds and was increased by blue light in einkorn and decreased by red light in both species. In addition, the lamp with the blue light and, to a lower extent, that with the red light resulted more efficient (as the ratio between energy emitted and electricity consumed, Watt:Watt) than the multi-wave lamp. Hence, using monochromatic LED light would represent an energy saving technique to produce sprouts and wheatgrass as micro-scale vegetables with high phenolic content.

Acknowledgements

We gratefully acknowledge GNC s.r.l. for providing the LED lamps, Mr. Massimo Fiorani (Prometeo s.r.l.) for providing grains, and Mr. Silvano Locchi and Mr. Rossano Cortona for technical assistance in the seed lab.

Funding sources

This research was supported by the Project "Ricerca di Base 2018-2020" of the Department of Agricultural, Food and Environmental Sciences of the University of Perugia (Coordinator: Paolo Benincasa).

References

Acosta-Estrada, B.A., Gutiérrez-Uribe, J.A., Serna-Saldívar, S.O., 2014. Bound phenolics in foods, a review. Food Chem. 152, 46–55.

Alrifai, O., Hao, X., Marcone, M.F., Tsao, R., 2019. Current review of the modulatory effects of LED lights on photosynthesis of secondary metabolites and future perspectives of microgreen vegetables. J. Agric. Food Chem. 67, 6075–6090.

Bantis, F., Smirnakou, S., Ouzounis, T., Koukounaras, A., Ntagkas, N., Radoglou, K., 2018. Current status and recent achievements in the field of horticulture with the use of light-emitting diodes (LEDs). Sci. Hortic. 235, 437–451.

Benincasa, P., Galieni, A., Manetta, A.C., Pace, R., Guiducci, M., Pisante, M., Stagnari, F., 2015. Phenolic compounds in grains, sprouts and wheatgrass of hulled and non-hulled wheat species. J. Sci. Food Agric. 95, 1795–1803. https://doi.org/10.3390/nu11020421.

Benincasa, P., Falcinelli, B., Lutts, S., Stagnari, F., Galieni, A., 2019. Sprouted grains: a comprehensive review. Nutrients 11, 421.

Bian, Z.H., Yang, Q.C., Liu, W.K., 2015. Effects of light quality on the accumulation of phytochemicals in vegetables produced in controlled environments: a review. J. Sci. Food Agric. 95, 869–877.

Correll, M.J., Kiss, J.Z., 2005. The roles of phytochromes in elongation and gravitropism of roots. Plant Cell Physiol. 46, 317–323.

Demotes-Mainard, S., Péron, T., Corot, A., Bertheloot, J., Le Gourrierec, J., Pelleschi-Travier, S., Crespel, L., Morel, P., Huché-Thélier, L., Boumaza, R., Vian, A., Guérin, V., Leduc, N., Sakr, S., 2016. Plant responses to red and far-red lights. applications in horticulture. Environ Exp Bot. 121, 4–21.

Dias, D.A., Urban, S., Roessner, U., 2012. A historical overview of natural products in drug discovery. Metabolites 2, 303–336.

Dykes, L., Rooney, L.W., 2007. Phenolic compounds in cereal grains and their health benefits. Cereal Foods World 52, 105–111.

Falcinelli, B., Benincasa, P., Calzuola, I., Gigliarelli, L., Lutts, S., Marsili, V., 2017a. Phenolic content and antioxidant activity in raw and denatured aqueous extracts from sprouts and wheatgrass of einkorn and emmer obtained under salinity. Molecules 22 (12), 2132. https://doi.org/10.3390/molecules22122132.

Falcinelli, B., Marconi, O., Maranghi, S., Lutts, S., Rosati, A., Famiani, F., Benincasa, P., 2017b. Effect of genotype on the sprouting of pomegranate (*Punica granatum* L.) seeds as a source of phenolic compounds from juice industry by-products. Plant Foods Hum. Nutr. 72, 432–438.

Falcinelli, B., Sileoni, V., Marconi, O., Perretti, G., Quinet, M., Lutts, S., Benincasa, P., 2017c. Germination under moderate salinity increases phenolic content and antioxidant activity in rapeseed (*Brassica napus* var *oleifera* Del.) sprouts. Molecules 22 (8), 1377. https://doi.org/10.3390/molecules22081377.

Falcinelli, B., Maranghi, S., Paoletti, A., Marconi, O., Rosati, A., Famiani, F., Benincasa, P., 2018. Sprouting olive (*Olea europaea* L.) seeds as a source of antioxidants from residual whole stones. Sci. Hortic. 240, 558–560.

Gan, R.Y., Chan, C.L., Yang, Q.Q., Li, H.B., Zhang, D., Ge, Y.Y., Gunaratne, A., Ge, J., Corke, H., 2019. Bioactive Compounds and Beneficial Functions of Sprouted Grains. In Sprouted Grains,Edition No. 1; Feng, H., Nemzer, B., DeVries, J.W., Eds. Woodhead Publishing and AACC International Press, Publisher, pp. 191–246.

Heo, J.W., Kang, D.H., Bang, H.S., Hong, S.G., Chun, C.H., Kang, K.K., 2012. Early growth, pigmentation, protein content, and phenylalanine ammonia-lyase activity of red curled lettuces grown under different lighting conditions. Korean J. Hortic. Sci. 30, 6–12.

Holopainen, J.K., Kivimäenpää, M., Julkunen-Tiitto, R., 2018. New light for phytochemicals. Trends Biotechnol. 36, 7–10.

Huché-Thélier, L., Crespel, L., Le Gourrierec, J., Morel, P., Sakr, S., Leduc, N., 2016. Light signaling and plant responses to blue and UV radiations-perspectives for applications in horticulture. Environ. Exp. Bot. 121, 22–38.

van Ieperen, W., 2012. Plant morphological and developmental responses to light quality in a horticultural context. Acta Hortic. 956, 131–139.

Iwai, M., Ohta, M., Tsuchiya, H., Suzuki, T., 2010. Enhanced accumulation of caffeic acid, rosmarinic acid and luteolin-glucoside in red perilla cultivated under red diode laser and blue LED illumination followed by UV-A irradiation. J. Funct. Foods 2, 66–70.

Khan, M.I.R., Fatma, M., Per, T.S., Anjum, N.A., Khan, N.A., 2015. Salicylic acid-induced abiotic stress tolerance and underlying mechanisms in plants. Front. Plant Sci. 6, 462. https://doi.org/10.3389/fpls.2015.00462.

Khanbabaee, K., Van Ree, T., 2001. Tannins: classification and definition. Nat. Prod. Rep. 18, 641–649.

Krygier, K., Sosulski, F., Hogge, L., 1982. Free, esterified, and insoluble-bound phenolic acids. 1. Extraction and purification procedure. J. Agric. Food Chem. 30, 330–334.

Lee, S.W., Seo, J.M., Lee, M.K., Chun, J.H., Antonisamy, P., Arasu, M.V., Suzuki, T., Al-Dhabi, N.A., Kim, S.J., 2014. Influence of different LED lamps on the production of phenolic compounds in common and Tartary buckwheat sprouts. Ind. Crop. Prod. 54, 320–326.

Liu, H., Chen, Y., Hu, T., Zhang, S., Zhang, Y., Zhao, T., Yu, H., Kang, Y., 2016. The influence of light-emitting diodes on the phenolic compounds and antioxidant activities in pea sprouts. J. Funct. Foods 25, 459–465.

Mert-Türk, F., 2002. Phytoalexins: defence or just a response to stress? J Cell Biol Mol Sci 1, 1–6.

Ouzounis, T., Razi Parjikolaei, B., Fretté, X., Rosenqvist, E., Ottosen, C.O., 2015. Predawn and high intensity application of supplemental blue light decreases the quantum yield of PSII and enhances the amount of phenolic acids, flavonoids, and pigments in Lactuca sativa. Front. Plant Sci. 6, 19. https://doi.org/10.3389/fpls.2015.00019.

Paterson, J.R., Lawrence, J.R., 2001. Salicylic acid: a link between aspirin, diet and the prevention of colorectal cancer. QJM: inter. J. Medicine. 94, 445–448.

R Core Team, 2014. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna. http://www.R-project.org/.

Rozema, J., van de Staaij, J., Björn, L.O., Caldwell, M., 1997. UV-B as an environmental factor in plant life: stress and regulation. Trends Ecol. Evol. 12, 22–28.

Salisbury, F.J., Hall, A., Grierson, C.S., Halliday, K.J., 2007. Phytochrome coordinates Arabidopsis shoot and root development. Plant J. 50, 429–438.

Samuolienė, G., Urbonavičiūtė, A., Brazaitytė, A., Šabajevienė, G., Sakalauskaitė, J., Duchovskis, P., 2011. The impact of LED illumination on antioxidant properties of sprouted seeds. Cent. Eur. J. Biol. 6, 68–74.

Samuoliene, G., Brazaityte, A., Vaštakaite, V., 2017. Light-Emitting Diodes (LEDs) for Improved Nutritional Quality. In: Dutta Gupta, S. (Ed.), Light Emitting Diodes for Agriculture, edition no. 1. Springer, Singapore, pp. 149–190.

Shimizu, H., 2016. Effect of light quality on secondary metabolite production in leafy greens and seedlings. In: Kozai, T., Fujiwara, K, Runkle, E.S (Eds.), LED Lighting for Urban Agriculture, Edition no. 1. Springer, Singapore, pp. 239–260.

Singleton, V.L., Rossi, J.A., 1965. Colorimetry of total phenolics with posphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic. 16, 144–158.

Son, K.H., Oh, M.M., 2013. Leaf shape, growth, and antioxidant phenolic compounds of two lettuce cultivars grown under various combinations of blue and red light-emitting diodes. HortScience 48, 988–995.

Stagnari, F., Galieni, A., D'Egidio, S., Falcinelli, B., Pagnani, G., Pace, R., Pisante, M., Benincasa, P., 2017. Effects of sprouting and salt stress on polyphenol composition and antiradical activity of einkorn, emmer and durum wheat. Ital. J. Agron. 12 (848), 293–301.

Taulavuori, E., Taulavuori, K., Holopainen, J.K., Julkunen-Tiitto, R., Acar, C., Dincer, I., 2017. Targeted use of LEDs in improvement of production efficiency through phytochemical enrichment. J. Sci. Food Agric. 97, 5059–5064.

Taulavuori, K., Pyysalo, A., Taulavuori, E., Julkunen-Tiitto, R., 2018. Responses of

phenolic acid and flavonoid synthesis to blue and blue-violet light depends on plant species. Environ. Exp. Bot. 150, 183–187.
Thwe, A.A., Kim, Y.B., Li, X., Seo, J.M., Kim, S.J., Suzuki, T., Chung, S.O., Park, S.U., 2014. Effects of light-emitting diodes on expression of phenylpropanoid biosynthetic genes and accumulation of phenylpropanoids in *Fagopyrum tataricum* sprouts. J. Agric. Food Chem. 62, 4839–4845.
Ti, H., Zhang, R., Zhang, M., Li, Q., Wei, Z., Zhang, Y., Tang, X., Deng, Y., Liu, L., Ma, Y., 2014. Dynamic changes in the free and bound phenolic compounds and antioxidant activity of brown rice at different germination stages. Food Chem. 161, 337–344.
Tosti, G., Benincasa, P., Cortona, R., Falcinelli, B., Farneselli, M., Guiducci, M., Onofri, A., Pannacci, E., Tei, F., Giulietti, M., 2017. Growing lettuce under multispectral light-emitting diodes lamps with adjustable light intensity. Ital. J. Agron. 11, 57–62.
Urbonavičiūtė, A., Samuolienė, G., Brazaitytė, A., Duchovskis, P., Ruzgas, V., Žukauskas, A., 2009a. The effect of variety and lighting quality on wheatgrass antioxidant properties. Zemdirbyste-Agriculture 96, 119–128.
Urbonavičiūtė, A., Samuolienė, G., Sakalauskienė, S., Brazaitytė, A., Jankauskienė, J., Duchovskis, P., Ruzgas, V., Stonkus, A., Vitta, P., Žukauskas, A., Tamulaitis, G., 2009b. Effect of flashing amber light on the nutritional quality of green sprouts. Agron. Res. 7, 761–767.
Xiao, Z.P., Peng, Z.Y., Peng, M.J., Yan, W.B., Ouyang, Y.Z., Zhu, H.L., 2011. Flavonoids health benefits and their molecular mechanism. Mini-Rev. Med. Chem. 11, 169–177.