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Productivity, nutritional and functional qualities of perennial wall-rocket: Effects of pre-harvest factors

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ABSTRACT

Perennial wall-rocket (*Diplotaxis tenuifolia*) belongs to the Brassicaceae family. It has spread worldwide due to its functional properties, and has long been known in folk medicine of native populations in areas of the Mediterranean and western Asia. In the past, *Diplotaxis tenuifolia* was a herbaceous plant harvested and consumed as a spontaneous herb, but today it is an important leafy vegetable in ready-to-use salads, with an increasing impact in the national and international vegetable retail markets. The leaves of perennial wall-rocket have valuable nutritional properties because of the amounts of antioxidant compounds they contain, in particular glucosinolates, vitamin C, as well as flavonoids, which make their consumption beneficial for preventing some types of cancer and cardiovascular disease. In the current review, major pre-harvest factors of *Diplotaxis tenuifolia* production, such as cropping systems, fertilization, as well as water management and irrigation, are addressed with respect to crop productivity and leaf quality.

Key words: cropping practices, *Diplotaxis tenuifolia*, functional foods, glucosinolates, phytochemicals, produce processing, ready-to-use salads

INTRODUCTION

The Mediterranean basin is a centre of plant diversity; its western part is the homeland of *Diplotaxis tenuifolia* (L.) DC, commonly recognized as “wild rocket” or “perennial wall-rocket”, which is a species belonging to the Brassicaceae family, tribe Brassicae, subtribe Brassicinae (Martínez-Laborde, 1996). Growing perennial wall-rocket is gaining an economic interest due its use in ready-to-use salads, a rising trend that allows to extend produce quality, freshness and shelf-life. According to Bonasia et al. (2017), *D. tenuifolia* cultivation has spread significantly in Italy during the past twenty years, covering around 4,000 ha of horticultural areas, a fact attributed to its being a succulent leafy vegetable.

Perennial wall-rocket, a diploid species (x = 11), goes through several growing cycles thanks to its adventitious buds disseminated on its roots.

D. tenuifolia is spread worldwide as a result of its adaptability and the ease of propagation; even its invasive behaviour has been considered as a case study (Hurka et al., 2003). Producing allelopathic substances, such as *S*-glucopyranosyl thiohydroximate, has helped it to develop great competitive ability in floristic communities

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(Giordano et al., 2005). In Italy, *D. tenuifolia* spontaneously thrives along coastal areas at altitudes below 400 m. As stated by Pignone (1996), a well-drained sandy-loam soil is adequate for wall-rocket cultivation; nevertheless, the plant is well-adapted to harsh and calcareous soils. It is worth mentioning that this leafy vegetable can grow even at a sodium chloride (NaCl) concentration of 300 mM, making it a highly salt-tolerant crop (De Vos et al., 2013). According to Hall et al. (2012), a temperature ranging from 2 to 25°C and an increase in day-length make the cool-season crop of perennial wall-rocket grow faster. New buds and shoots emerge at the plant's base thanks to the storage and utilization of carbohydrate reserves in taproot, leading to plant regrowth upon leaf cutting done at 3-5 cm above soil surface (Erice et al., 2011). In a recent review article, Caruso et al. (2018) covered several aspects that might affect the yield and quality of perennial wall-rocket, including the aspects of crop protection against particular pests, pathogens and diseases, as well as post-harvest aspects, whereas the information in the scientific literature still does not include pre-harvest factors. Therefore, the present review focuses on recent advances in the study of perennial wall-rocket, in particular those pertinent to pre-harvest factors such as cropping systems, fertilization, and irrigation, which are addressed with respect to crop productivity and leaf quality.

FARMING MANAGEMENT

Cropping systems

Perennial wall-rocket is cultivated both under open-field conditions and especially under protected cultivation in either soil or soilless systems. Under protected cultivation the production can be planned all year round by performing a higher number of crop cycles compared to open-field cultivation, and additionally getting a cleaner produce with no damage due to lodging caused by rain or hail, and with a better nutrient solution management coupled with a better crop protection. Indeed, most of the cultivation is carried out in tunnel-shaped greenhouses with a span of 5 to 10 m and volume ranging from 1.5 to 5.0 m^3 per square meter of soil surface, covered with polyethylene films having a thickness of 0.15 to 0.25 mm. Sometimes these protective structures are equipped with anti-freeze systems, such as an additional polyethylene film or non-woven polypropylene fabric laid on plants or above greenhouse roof irrigation to prevent irreversible damage to plants. Actually, the optimal minimum thermal values for rocket (16-18°C at night) can seldom be reached during late autumn to early spring cycles in temperate areas. Optical features of the greenhouse cover play an essential role with regard to both thermal and light conditions affecting produce quality. Notably, inadequate light transmissivity of the cover film may further reduce the low solar radiation intensity during the winter season, causing leaf etiolation, light green colouring with elongated petioles, poor intensity of aroma, high nitrate content and poor shelf-life (Di Benedetto and Giordano, 2011). Moreover, excessive humidity inside the greenhouse can encourage the spreading of fungal diseases (Di Benedetto and Giordano, 2011).

The cropping system (open-field or greenhouse) can significantly affect crop productivity of perennial wall-rocket. In this respect, Hall et al. (2012) conducted a series of experiments under open-field conditions, reporting that yield is correlated with the harvest season and the number of cropping cycles. In fact, in their study, the highest fresh yield was obtained in both the 'European wild rocket' and 'Appolo' cultivars for the first harvest of the summer crop (2.7 and 2.8 kg m^{-2}, respectively), whereas with regard to the second harvest in the summer and spring seasons the cultivar 'Nature' produced the highest yield (2.5 kg m^{-2}). Similarly, Bonasia et al. (2017) showed that soilless-grown cultivar 'Naturelle' provided a higher fresh yield (+30%) compared to 'Nature', irrespective of whether the cropping cycle occurred in autumn-winter or in winter-spring. The authors associated the better crop performance to a higher nitrogen use efficiency of the genetic material (Bonasia et al., 2017).

In hydroponics, where both fertilization and growing conditions are efficiently managed, the production is reliably planned and more uniform leaves are obtained. In Italy, the most used soilless technique for growing *D. tenuifolia* is the floating raft system, which reduces foliar diseases and weed contamination, leading to a clean produce requiring only washing before being packed. Under that type of crop management, a 29.6% increase in the yield of fresh leaves was recorded as a result of doubling the plant density in the autumn-winter cycle (Nicola et al., 2005). In another investigation carried out on rocket grown in a floating system with 1,150 plants m^{-2}, the 1.83 dS m^{-1} EC of nutrient solution in summer resulted in the highest production (1,901 g m^{-2}), whereas no significant effect of the 1.51 to

2.63 dS m^{-1} EC range was recorded in autumn crops (Alberici et al., 2008).

Planting time and density

Sowing is performed from early autumn to spring for crops intended for the "baby leaf produce" industry, whereas transplanting is preferred for fresh market crops, and it takes place in the same periods mentioned for sowing (Caruso et al., 2011).

Bonasia et al. (2017) recorded higher leaf yields of perennial wall-rocket in the winter-spring cycle than in autumn-winter (2.25 vs. 1.50 kg m^{-2}). The highest crop performance in terms of fresh yield was attributed to the optimal climatic conditions inside the greenhouse, in particular air temperature and global radiation. In fact, during the winter-spring growing season, air temperature was always over 20°C and less frequently below 5°C, with greater day-length and solar radiation, the latter being 36% higher in winter-spring than in autumn-winter (429.3 vs. 315.3 MJ m^{-2}).

Before planting and at adequate soil humidity, the soil is cultivated to a depth of 30 cm, starting by ploughing or spading, and periodical deep tillage then harrowing, followed by surface levelling and, finally, the formation of ridges (1-2 m wide) done prior or during sowing. As good agricultural practices, application of green manure along with solarization are worth mentioning, whereas the removal of crop residues is crucial in order to prevent a pathogen threat. Although crop rotation is beneficial, rocket can have probable allelopathic effects on species from the following families: Papilionaceae, Apiaceae, Cucurbitaceae and Solanaceae.

When "baby leaf" is the target produce, bare soil is cultivated and mechanical sowing is performed using self-moving or tractor-mounted sowers, usually as large as the harvesting machines in order to optimize the work (Di Benedetto and Giordano, 2011). This agricultural practice is performed arranging continuous rows 5 cm apart with seeds placed at a depth of 3-5 mm, with a plant density ranging from 1,800 to 2,800 plants m^{-2} using 7 to 8 kg of seeds ha^{-1} respectively in winter and summer production (Schiattone et al., 2018). Excessive planting densities, however, result in pale, tender leaves unsuitable for handling and storage, in addition to a pathogen infection risk due to high humidity, and a nitrate increase caused by low light intensity (Di Benedetto and Giordano, 2011). To avoid compressing the soil excessively, formation of ridges, surface levelling and sowing are carried out in one single operation.

When fresh market is the destination of the produce, the ridges are mulched with black polyethylene or biodegradable film, which shortens the crop cycle, controls weeds and pests, and finally improves produce quality; under the mulch, drip pipes are placed in alternate rows. In this cropping system, multi-seeded cells (15-25 seeds per cell) are transplanted, after rearing 3-leaf plantlets for 20-30 days in multi-cell trays filled with a peat-vermiculite medium (Di Benedetto and Giordano, 2011). As stated by Pimpini and Enzo (1996), the inter-row spacing is 20 cm, while the intra-row spacing is 10-15 cm, resulting in a plant density of 500-1,250 plants m^{-2}, or even more (Nicola et al., 2005). Doubling plant density from 1,067 to 2,134 plants m^{-2} in *D. tenuifolia* made it possible to obtain an increase of 29.6% in leaf fresh weight under a soilless system (1,684 and 2,182 g m^{-2}, respectively) and a 22.5% increase under soil-bound cultivation (443 and 543 g m^{-2}, respectively) (Nicola et al., 2005).

Nutritional requirements and fertilization

A not-too-high nutritional input is necessary for growing perennial wall-rocket; as reported by Pimpini et al. (2005), a 100 kg yield requires 0.28 kg N, 0.11 kg P_2O_5 and 0.34 kg K_2O. Either traditional fertilization or fertigation can be applied, where $1500 < EC < 2500$ µS cm^{-1}, $6 < pH < 6.5$, and N, P_2O_5 and K_2O at the following ratio: 1.5:0.5:1.0 during the first growing cycle, and at 2.0:0.5:1.5 for the subsequent ones. As stated by several authors (Bianco and Boari, 1996; Pimpini and Enzo, 1996), nitrogen supply should not exceed 100 kg ha^{-1} in open-field spring-summer planting, and 200 kg ha^{-1} in protected environment and sandy soil when several growing cycles are aimed at. Indeed, *D. tenuifolia* has a short growing cycle and, in addition, nitrate accumulation is high, up to 10 g kg^{-1} leaf fresh weight (Santamaria et al., 2001; Caruso et al., 2011), since it has a low nitrogen use efficiency (Santamaria et al., 2002). Moreover, no nitrogen fertilization should be applied close to harvest time, especially in hydroponics, because an excess of this nutrient can reduce product quality during storage. However, produce yield and quality are affected by nitrogen and water availability (Hu and Schmidhalter, 2005).

As reported by Schiattone et al. (2018), the values of nitrogen uptake and nitrogen use efficiency (NUE), as average values of all treatments, ranged among the various crop cycles from 3.1 to 5.8 g m^{-2} and from 24.5 to 25.8 g g^{-1} DM, respectively. Nitrogen uptake response in relation to irrigation replenishment (IR) varied among the crop cycles. In the first one, no differences among irrigation treatments were observed, in the second and third ones I75 (replenishment of 75 % evapotranspirated water) gave the lowest values, in the fourth crop cycle, instead, the lowest values were observed at I75 and I150. In all the crop cycles, I75 showed a 13.7% higher NUE than the other irrigation treatments. N uptake and NUE, with the exception of the first crop cycle, in which no differences between nitrogen levels were observed, were respectively lower (by 21.5%) and higher (by 14%) in the 60 kg ha^{-1} N treatment compared to 120 kg ha^{-1} N (Schiattone et al., 2018).

The harvesting time of *D. tenuifolia* cannot be established in advance because it has a variable crop cycle duration, depending on climatic conditions, fresh market demand and phytosanitary status, factors that also control the feasibility of several harvests. Nicola et al. (2005) reported a 70-day interval from late-autumn seeding to mid-winter harvest in soilless-grown rocket, whereas a longer crop cycle was recorded in traditional (soil-bound) cultivation.

Rocket leaves can be cut manually or by mowers when they have reached a height of 10-15 cm (Alberici et al., 2008), and the most appropriate timing must be decided according to local conditions: a morning harvest, under lower temperature and higher relative humidity, is preferable in the spring and summer; afternoon picking is suggested in case of a high nitrate concentration concern, especially because perennial wall-rocket is a hyper-accumulator species (Caruso et al., 2011). At harvest, the fresh product is placed in plastic boxes to be carried to the processing plant. For the preparation of ready-to-use salads, leaves must be refrigerated and conditioned in due time to ensure an adequate shelf-life.

Using the nutrient film technique, the highest perennial wall-rocket yield (12.5 t ha^{-1}) was obtained with 122 mg dm^{-3} N and with an electrical conductivity (EC) of 2.2 dS m^{-1} (Cavarianni et al., 2008); moreover, the cultivar ‘Cultivada’ produced the highest yield (16.9 t ha^{-1}), followed by ‘Folha Larga’ and finally ‘Selvática’ (12.3 and 2.9 t ha^{-1}, respectively).

Bonasia et al. (2017) reported that in the autumn-winter cycle the increase in nutrient solution concentration from 2.5 to 3.5 dS m^{-1} EC caused a significant reduction in leaf fresh yield (–18.7%), whereas in the winter-spring growing cycle, increasing the EC of the nutrient solution from 3.5 to 4.5 dS m^{-1} resulted in a milder reduction in fresh leaf yield (–10.3%).

Water management

Having a 40 cm deep taproot system makes perennial wall-rocket a species adaptable to harsh and arid soils, thus reducing irrigation requirements and preventing fungal diseases caused by excessive irrigation. Nevertheless, a good irrigation provision ensures succulent, high quality leaves with less undesirable fibres.

Bianco (1995) reported that a water shortage decreases leaf number and size per plant, making the yield and produce quality of a *Diplotaxis tenuifolia* crop strongly correlated with water availability, which is in accordance with Stefanelli et al. (2010), who found that antioxidant content is negatively affected by water shortage.

Water consumption of perennial wall-rocket varies between 35.1 mm and 48.2 mm in autumn and spring respectively, and watering frequency also increases from 3 times in winter to 5 times in autumn (Schiattone et al., 2018). Usually, irrigation with sprinklers is used for industrial crops, with a flow ranging from 110 to 205 L h^{-1} and 0.05 to 0.1 nozzle per m^2 (3 × 3 m). Such irrigation is also useful for plant protection treatments, placed at 2 m above the soil to facilitate the movement of machines. Otherwise, the drip irrigation method with a flow rate of 2-4 L h^{-1} is normally used for fresh market produce.

Schiattone et al. (2018) reported that a water supply of 100% of crop evapotranspiration (ETc) was the best irrigation regime for marketable fresh yield, amounting to 1.51 kg m^{-2} as the average of four crop cycles. The same authors reported that irrigation with 75%, 125% and 150% of crop evapotranspiration resulted in lower marketable yields compared to 100% ETc, by about 8%, 4% and 6%, respectively. Specifically, in the second crop cycle a yield reduction of 5% for 75% and 150% ETc was recorded, in the third crop cycle a reduction of 9% for 75% ETc, and in the fourth crop cycle 18%, 10% and 15% reduction corresponding to 75%, 125% and 150% ETc, respectively. The average plant weight and the number of leaves per plant mainly contributed to the differences in yield among the previous irrigation treatments. The highest N dose (120 kg ha^{-1}) resulted in a 13% higher yield (average of four crop cycles 1.53 kg m^{-2}) compared to the lowest dose (60 kg ha^{-1}). This beneficial effect was more pronounced in the second (+14%), third (+18%) and fourth (+16%) crop cycle. However, in the third, and especially in the fourth, crop cycle, an adverse N effect on fresh yield, on leaf area index (LAI) as well as on plant mean weight of less irrigated crops was observed. Indeed, in the 75% ETc treatment, in contrast to the other irrigation levels, the yield, LAI and plant mean weight decreased with the increase in N level. Finally, in the second and third crop cycle, the variations in average plant weight and plant leaf number mainly contributed to the changes in marketable yield in relation to the N rate (Schiattone et al., 2018).

The values of yield water use efficiency (YWUE), biomass water use efficiency (BWUE) and irrigation yield water use efficiency (IYWUE) among the various crop cycles ranged from 33.0 to 37.3 kg m^{-3}, from 4.5 to 7.3 kg m^{-3}, and from 31.4 to 35.0 kg m^{-3}, respectively. Yield WUE response in relation to irrigation was significantly different among the different crop cycles. In the first crop cycle, this parameter was 23.4% higher in the 75% ETc treatment compared to the other irrigation regimes, whereas in the second crop cycle a higher value (by 8.5 % on average) was recorded in the 75%, 100% and 125% ETc treatments compared to 50%. In the third crop cycle, the situation was reversed, with higher values of YWUE at 100%, 125% and 150% ETc. Finally, in the last crop cycle, the highest value was observed in the 100% and 125% ETc treatments. Similar to YWUE, the BWUE and IYWUE decreased when changing from 75% to 150% ETc in all the crop cycles, with the average values being halved. As reported by Schiattone et al. (2018), the yield, biomass and irrigation yield WUE, with the exception of the first crop cycle, in which no differences were observed, were higher by 16.1%, 2.6% and 14.0%, respectively, under the high application rate of N (120 kg ha^{-1}) compared to the moderate N application rate (60 kg ha^{-1}).

Growth, harvest and yield

It is well established that leaf area index of perennial wall-rocket ranges from 1.9 in autumn to 3.3 in winter, whereas plant weight ranges from 1.9 g per plant in autumn to 8.4 g per plant in spring; leaf number increases from 4.6 per plant in autumn to 16.2 per plant in spring. Moreover, total length and length of leaf blade range from 14.8 cm to 5.7 cm in autumn and from 20.1 cm to 10.3 cm in spring, respectively, with a percentage ratio of the two parameters ranging from 39.1% to 53.5% in autumn and winter, respectively; leaf width varies from 18.4 mm in autumn to 10.4 mm in winter; leaf incision index increases from 2.9 in autumn to 5 in winter-spring (Schiattone et al., 2018).

Under optimal pedo-climatic conditions, up to 5-6 *Diplotaxis tenuifolia* cropping cycles can be performed, as reported by Bianco (1995), and each of them lasts 20-100 days, depending on the season and product destination (Pimpini and Enzo, 1996; Martínez-Sánchez et al., 2008; Schiattone et al., 2018). Harvest generally takes place before the emergence of inflorescence, under moderate temperatures in order to avoid cell turgor loss, and the fresh leaves undergo industrial processing upon very fast transport from fields to factories by means of refrigerated lorries.

Manual harvesting is practised when the produce is destined for the fresh vegetable market, and this work can be performed by means of knives or sickles and collecting plates. Otherwise, industrial crops are harvested mechanically with self-moving machines whose heads are equipped with a rotating blade-belt and a carpet, even acting as a shaker in more advanced machines, conveying the leaves into a plastic bin. Immediately after harvesting, plant residues are removed from the field by means of a suction machine to prevent them from contaminating the product of later harvests. As reported by Schiattone et al. (2018), plants are cut 3 to 5 cm above the cotyledons to allow efficient vegetative apex regrowth. The number of possible harvests of perennial wall-rocket changes depending on the season, with marketable yield varying between 5 and 18 t ha^{-1} per cycle and overall exceeding 60 t ha^{-1}. In the autumn-spring cycle, up to 5 harvests can be obtained with 10 to 15 cm long leaves, while in spring-summer fewer harvests can be planned, as the plants rapidly grow and switch to the reproductive phase under the long photoperiod (Schiattone et al., 2018).

LEAF QUALITY AND NUTRACEUTICAL PROPERTIES

Besides the pressing issue of obtaining high fresh yields to meet global food security, demand for high quality green leaves is also on the rise, driven by the growing interest of society in fresh products of high nutritional and functional quality. The quality of fresh horticultural species, including leafy vegetables, has been recently defined as '*a dynamic composite of their physicochemical properties and evolving consumer perception, which* *embraces organoleptic, nutritional and bioactive components*' (Kyriacou and Rouphael, 2018). *D. tenuifolia* produce depreciates when leaf petioles are excessively long compared with the blades, and the colour intensity is not consistent with consumer requirements; in this respect, genetic material plays a crucial role, as drawn by cultivar comparisons where 'Naturelle' showed greener leaves than 'Nature' irrespective of the growing seasons and agricultural practices (Bonasia et al., 2017).

As reported by Delaveau and Paris (1958), the bitter or pungent taste of perennial wall-rocket leaves is caused by glucosinolates, while enzymatic hydrolysis of these compounds produces volatile isothiocyanates that are responsible for the strong acrid aroma.

Glucosinolates are sulfur-containing compounds found in the Brassicaceae family; they are a class of β-thioglucoside-N-hydroxysulfates (Fahey et al., 2001). *D. tenuifolia* contains a high concentration of 4-mercaptobutylglucosinolate (glucosativin), but a lower concentration of 4-methylthiobutyl-glucosinolate (glucoerucin) and 4-methylsulfinyl-butylglucosinolate (glucoraphanin); moreover, 4-hydroxybenzylglucosinolate (sinalbin) is mainly present in the roots (Bennett et al., 2006, 2007), whereas 3-butenylglucosinolate (gluconapin) and some indolylglucosinolates are minor compounds (Nitz and Schnitzler, 2002). The glucosinolate content of perennial wall-rocket leaves is controlled mainly by genetic factors, but also by cropping practices; indeed, the levels of glucoerucin and glucoraphanin, and accordingly the physiological nutrient value, are higher in successive harvests (Nitz and Schnitzler, 2002).

Bonasia et al. (2017) reported that the most abundant glucosinolates in *D. tenuifolia* leaves obtained in soilless cultivation are glucoraphanin, both in autumn-winter and winter-spring cycles, and glucoerucin and aliphatic proidrin in winter-to-spring cultivation; moreover, aliphatic glucobrassiconapoleiferin, glucobrassiconapin, and indolic 4-hydroxy glucobrassicin (4-OH) build up only in winter-spring, whereas indolic glucobrassicin in both cycles. In addition to the effect of the growing season, the genetic material can significantly affect the glucosinolate profile. Bonasia et al. (2017) also demonstrated that 'Naturelle' grown in a floating raft system exhibited higher values of glucoraphanin, glucoerucin and epiproidrin in the autumn-winter cycle, supporting previous studies suggesting that the glucosinolate profile is genotype-dependent (Bennett et al., 2006; D'Antuono et al., 2009; Bell et al., 2015). 'Naturelle' also showed the highest aliphatic proidrin /epiproidrin ratio, which is strictly correlated with leaf bitterness and pungency responsible for the exclusive taste of rocket salad (*Diplotaxis* and *Eruca* spp.) (Pasini et al., 2011).

Perennial wall-rocket leaves are categorized high among salads due to their richness in dry matter fibre, macrominerals as well as vitamins (Bruno et al., 1980; Pimpini et al., 2005).

Thicker leaves, i.e. higher dry matter percentage (10.4% vs. 8.3%) and specific leaf area (34.8 vs. 24.2 g cm^{-2}), were obtained in the winter-spring cycle compared with the autumn-winter one in soilless-grown crops (Bonasia et al., 2017); however, the leaves harvested in spring had more damaged membranes and dehydrated tissues compared to winter leaves (membrane efflux of electrolytes 17.9% vs. 11.8%) as well as a lower relative water content (64.5% vs. 79.6%). Moreover, higher values were recorded in a floating than in an ebb-and-flood soilless system in terms of dry weight percentage (89.9% vs. 76.5%, respectively) and specific leaf area (+13.3%), resulting in thicker leaves, as well as chlorophyll content on a dry weight basis (+13%), total carotenoids, vitamin C, total glucosinolates, total phenols, total antioxidant capacity, and hydrophilic antioxidant capacity. In the autumn-winter cycle, the increase in nutrient solution concentration from 2.5 to 3.5 dS m^{-1} EC caused an increase in dry matter concentration (+21.3%), specific leaf area (+21.0%), chlorophyll content (+23%), leaf green colour, total carotenoids, total phenols, vitamin C, hydrophilic and total antioxidant capacity, aliphatic epiproidrin, gluco-erucin and indolic glucobrassicin; in the winter-spring cycle, the increase in EC from 3.5 to 4.5 dS m^{-1} caused a reduction in vitamin C and total glucosinolates (Bonasia et al., 2017). Ascorbic acid synthesis was promoted by an EC close to 3.5 dS m^{-1} as a consequence of the response to the osmotic stress caused by salinity (Guo et al., 2013), whereas higher salinity levels may induce excessive stressful conditions, resulting in an irreversible hydrolization of the de-hydro-ascorbic acid form (Gallie, 2013), i.e. an inhibition of the activity of ascorbic acid regenerating enzyme. The significant glucosinolate decrease from 3.5 to 4.5 dS m^{-1} EC is mainly due to the decrease in glucoraphain, which is the most abundant glucosinolate in rocket and degrades to isothiocyanate sulphoraphane (Guo et al., 2013); the latter compound is supposed to balance the vitamin C reduction, thus contributing to the stability of total antioxidant capacity upon the 3.5 to 4.5 dS m^{-1} EC increase.

Bonasia et al. (2017) found higher concentrations of antioxidants in the leaves of soilless-grown wild-rocket harvested in spring than in winter, such as vitamin C (239.0 vs. 152.7 mg kg^{-1} FW), total phenols (997 vs. 450 mg GAE mg kg^{-1} FW), and total glucosinolate concentration (1,078.8 vs. 405.7 mg kg^{-1} DW), with only the carotenoids being lower (7.7 vs. 12.8 mg 100 g^{-1} FW). Consequently, a higher total antioxidant capacity was detected (11.5 vs. 8.6 µmol Trolox eq. kg^{-1} FW), mainly due to the hydrophilic components (10,939 vs. 8,462 µmol Trolox eq. kg^{-1} FW), strongly linked to total phenols, glucosinolates and vitamin C.

As reported by Alberici et al. (2008), no differences in total concentrations of phenols in rocket leaves were generally found among seasons, whereas the content of these compounds was significantly affected by nutrient solution EC and in fact it increased from 1.51 to 2.63 dS m^{-1}. Moreover, the highest amount of carotenoids was found in the spring-summer cycle (982 µg cm^{-2} on average); in September it attained the top value under the 2.63 dS m^{-1} EC (995 µg cm^{-2}). Rocket leaves showed the highest chlorophyll concentration in the June harvest (22.3 µg cm^{-2}); it increased from 1.51 to 1.83 dS m^{-1} nutrient solution EC in September (from 18 to 20 µg cm^{-2}).

Total phenols and total antioxidant activity increased from the first to the last crop cycle, and in water as well as nutritional stress conditions (Schiattone et al., 2018). Total phenolic content was higher at 75% water replenishment compared to other water replenishment treatments (about +20%), and at the lowest N rate of 60 kg N ha^{-1} (more than 9%), whereas total antioxidant activity was higher by about 23% and 11% in the treatments with water limitation and with the lowest N rate, respectively. Irrigation regimes affected chlorophyll content in the third and fourth crop cycle, with the lowest values at 75% and 150% water replenishment; moreover, this parameter was by 9% higher at 120 kg N ha^{-1} compared to 60 kg N ha^{-1} (Schiattone et al., 2018).

Carotenoid content increased progressively from the first to the last crop cycle and decreased with the increase in water availability in the first two crop cycles. In later crop cycles, this parameter was higher at I75 (replenishment of 75% evapo-transpirated water) and I150; in addition, a 10% higher carotenoid content was found at the lowest N rate of 60 kg N ha^{-1} (Schiattone et al., 2018). Antioxidant properties are also characteristic of the alkaloid sinapine (4-hydroxy-3,5-dimethoxy-cinnamic acid choline ester) contained in *D. tenuifolia* seeds, which, however, is better known as an anti-nutritional factor (Boucherau et al., 1991; Bennett et al., 2006).

A potential pharmaceutical interest relies on some compounds that have been recently extracted from perennial wall-rocket leaves, such as some nortropane alkaloids derived from pseudotropine, known as calystegins, especially calystegin A5 (Brock et al., 2006), and an essential oil containing 5-methylthiopentanenitrile possessing antifungal properties (Rodriguez et al., 2006).

Nitrogen availability in leafy vegetables affects many qualitative parameters, such as the amounts of dry matter, nitrate, vitamin C and polyphenols, and also leaf green colour, crunchiness, and shelf-life (Maggio et al., 2013). Adverse relationships were found between the N level and phenols, glucosinolates and vitamin C content, but a positive relationship with chlorophyll content (Stefanelli et al., 2010).

Schiattone et al. (2018) reported that DM increased from the first (7.3 g 100 g^{-1} FW) to the successive crop cycles (averaging 8.7 g 100 g^{-1} FW). Leaf nitrate concentration decreased progressively from an average value of 5,250 mg kg^{-1} FW of the 1st crop cycle to 2,321 mg kg^{-1} FW of the last one. Irrigation regimes did not lead to significant variations in leaf nitrate concentration in the first two crop cycles, whereas a contrasting effect was noted in the third and fourth crop cycle: in the third one, I75 resulted in an increase in nitrate concentration of approximately 23%, while in the following crop cycle a progressive increase occurred with increasing water supply, i.e. from 1,974 mg kg^{-1} FW at I75 to 2,658 mg kg^{-1} FW at I150. The highest N rate of 120 kg N ha^{-1} produced a more than 33% higher nitrate concentration than the lowest one.

The leaves of *Diplotaxis tenuifolia* plants grown in soil contained 41.5% more dry matter than the plants grown in soilless cultures (Nicola et al., 2005). Moreover, the leaves of soil-grown plants contained 37.9% less nitrate than those of soilless-grown ones (279 vs. 449 mg kg^{-1} FW), both much lower than expected in the winter season, when the nitrate reductase activity is reduced. However, nitrate accumulation was inversely correlated with the accumulation of organic compounds, suggesting low N assimilation efficiency.

Harvesting in the afternoon, after the plant has been exposed to a fairly long period of sunlight, resulted in leaves with a much lower nitrate concentration than in those harvested in the morning (Santamaria et al., 2001). Alberici et al. (2008) found that the leaf nitrate content of rocket was affected neither by the crop season nor by nutrient solution EC (2,800 mg kg^{-1} FW on average). When the nutrient film technique was used, leaf nitrate concentration was highest (2,410 mg kg^{-1} FW) under 244 mg N L^{-1} and 2.5 dS m^{-1} EC (Cavarianni et al., 2008). The cultivar 'Selvática' showed the highest nitrate content (1,859 mg NO_3^- kg^{-1} FW) in comparison with 'Folha Larga' and 'Cultivada' (1,035 mg NO_3^- kg^{-1} FW on average).

Bonasia et al. (2017) reported that the increase in EC from 2.5 to 3.5 dS m^{-1} due to the presence of NaCl resulted in a decrease in nitrate leaf concentration (–47%) in the autumn-winter cycle, whereas in winter-spring the 4.5 dS m^{-1} EC level did not reduce the nitrate content compared with the 3.5 dS m^{-1} EC, although the general climatic conditions of the period were more favourable for nitrogen assimilation (Blom-Zandstra, 1989; Colla et al., 2018), which may have been due to the higher chloride concentration inhibiting the activity of the nitrate reductase enzyme (Barber et al., 1989; Rouphael et al., 2018a,b). Moreover, leaf nitrate accumulation is also affected by the genetic material, as 'Naturelle' showed a lower content than 'Nature', suggesting that under non-saline conditions 'Naturelle' uses nitrogen more efficiently than 'Nature', accounting for its better productive performance and improved sensorial, physiological and nutritional characteristics (Bonasia et al., 2017).

CONCLUSIONS

Perennial wall-rocket is being increasingly grown as a potential functional species due to its climatic resilience as well as interesting yield performances and worthy nutritional properties. The latter are connected to the enhanced presence of valuable bioactive compounds, such as glucosinolates conferring the typical pungent taste favourably impacting on consumers, and antioxidants enriching the nutraceutical pattern. Modulating the pre-harvest factors such as genetic materials and agricultural practices, in particular cropping systems, fertilization and irrigation, can be an effective means of improving productivity and quality of the produce. Future experimental studies should focus on elucidating the genotype × environment × management interaction in order to assess various combinations and recommend the best ones to growers and extension specialists. Finally, research should also focus on the physiological and molecular modes of action responsible for increasing the levels of the perennial wall-rocket bioactive compounds under both open-field and greenhouse conditions.

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AUTHOR CONTRIBUTIONS

G.C. and Y.R. – conceived the review and contributed to bibliographic search as well as manuscript writing; S.DeP. – made a contribution in structuring and critically commenting on the manuscript; R.N. – was involved in bibliographic search and manuscript writing; E.C. – contributed to bibliographic search.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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