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Mini review

Rocket salad: crop description, bioactive compounds and breeding perspectives

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Abstract: **Rocket salad is a plant member of the Brassicaceae family whose name encloses species of the *Eruca* and *Diplotaxis* genera characterized by leaves with peculiar pungent taste and strong flavour. It has been originated in the Mediterranean area and nowadays is worldwide cultivated and consumed as food condiment and in ready-to-use mixed salad packages. Several other uses are recognized in cosmetics and medicine. This crop represents a valuable source of health benefits due to the presence of a range of health-promoting phytochemicals including carotenoids, vitamin C, fiber, polyphenols, and glucosinolates. These compounds are potentially linked in the prevention of certain diseases and types of cancer. Glucosinolates, represent the major class of compounds in rocket, and their hydrolysis products are responsible of the typical pungent aromas and flavours. Despite the continuous increase of the global consumption during the recent years, few efforts have been carried out in genetic improvement programs aimed to constitute new varieties due to biological and reproductive barriers. In the present article is provided a brief overview of the principal species of rocket salad used in dietary and discussed the qualitative properties as well as the potentiality and constraints for breeding.**

1. General Aspects

Rocket salad also known as arugula is an annual herbaceous plant whose name encloses several species of the Brassicaceae family characterized by leaves with peculiar pungent taste and strong flavour. The crop has been originated in the Mediterranean and Near East, with a major centre of diversity in the regions of Western Mediterranean (Hall *et al.,* 2015), which represent also the main areas of cultivation thanks to their growing conditions and climate.

Several species, mainly belonging to the genus *Eruca* and *Diplotaxis*, are widely cultivated and recognized as rocket salad. The most common are *Eruca vesicaria* (L.) Cav. and *Diplotaxis tenuifolia* (L.) Dc. *Eruca vesicaria* includes four subspecies namely subsp. *vesicaria*, subsp. sativa (Miller), subsp. *longirostris* (Uechtr.) and subsp. *pinnatifida* (Desf.) (Gomez-Campo, 2003). Among these, the subsp *sativa,* also called *Eruca sativa*, has been spreaded in different part of the world as cultivated rocket and is the most consumed and economically relevant. This species is diploid with eleven chromosomes (2n = 22) (Padulosi and Pignone, 1996) with annual life cycle flowering at begin of spring and ending with the production of seeds in late spring/early summer. Nowadays it is cultivated in all continents in both marginal areas and/or fertile soils. Plants of this species are characterized by a height of about 15.0 cm, flowers with calyx caduceus, sepals only two cucullate and corolla cream or whitish (Gomez-Campo, 2003).

Diplotaxis genus includes about 33 species (www.theplantlist.org) with great variability related to morphological traits and chromosome number (Table 1). The genus includes both annual and perennial plants with leaves of different shape, thickness,

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Table 1 - List of *Diplotaxis* species recognized and their chromosome number

Species	Chromosome number
D. acris (Forsk.) Boiss.	
var. *acris*	11
var. *duveyrieriana* (Coss.) Coss.	*na* *
D. antoniensis Rustan	*na*
D. assurgens (Del.) Thell.	9
D. berthautii Br.-Bl. and Maire	9
D. brachycarpa Godr.	9
D. brevisiliqua (Coss.) Mart.-Laborde	8
D. catholica (L.) DC.	
var. *catholica*	9
var. *rivulorum* (Br.-Bl. And Maire) Maire	*na*
D. erucoides (L.) DC.	
subsp. *Erucoides*	7
subsp. *longisiliqua* (Coss.) Gómez-Campo	7
D. glauca (J.A. Schmidt) O.E. Schulz	13
D. gorgadensis Rustan	
subsp. *gorgadensis*	*na*
subsp. *brochmanii* Rustan	*na*
D. gracilis (Webb) O.E. Schulz	13
D. griffitthii (Hook.f. and Thomps.) Boiss.	*na*
D. harra (Forsk.) Boiss.	
subsp. *crassifolia* (Rafin.) Maire	13
subsp. *harra*	13
subsp. *lagascana* (DC.) O. Bolòs and Vigo	13
subsp. *confusa* Mart.-Lab.	13
D. hirta (Chev.) Rustan and Borgen	13
D. ibicensis (Pau) Gómez-Campo	8
D. ilorcitana (Sennen) Aedo et al.	8
D. kohlaanensis A. Miller and J. Nyberg	*na*
D. muralis (L.) DC.	
subsp. *ceratophylla* (Batt.) Mart.-Laborde	*na*
subsp. *muralis*	21
D. nepalensis Hara	*na*
D. ollivierii Maire	*na*
D. pitardiana Maire	*na*
D. scaposa DC.	9
D. siettiana Maire	8
D. siifolia Kunze	
subsp. *bipinnatifida* (Coss.) Mart.-Laborde	*na*
subsp. *Siifolia*	10
subsp. *vicentina* (Samp.) Mart.-Laborde	10
D. simplex (Viv.) Sprengel	11
D. sundingii Rustan	13
D. tenuifolia (L.) DC.	
subsp. *cretacea* (Kotov) Sobr. Vesp.	11
subsp. *tenuifolia*	11
D. tenuisiliqua	
subsp. *rupestris* (J. Ball) Mart.-Laborde	*na*
subsp. *tenuisiliqua*	9
D. varia Rustan	*na*
D. villosa Boulos and Jallad	*na*
D. viminea (L.) DC. var. viminea and var. integrifo-	10
D. virgata (Cav. DC.)	
subsp. *sahariensis* Coss.	*na*
subsp. *virgata*	9
subsp. *rivulorum* (Br.-B1. and Maire) Mart.-	*na*
subsp. a*ustralis* Mart.-Lab.	*na*
D. vogelii (Webb) O.E. Schulz	*na*

* na = not available.

indentation, and flower colour (white, yellow and purple). The most common species cultivated across all continents are *Diplotaxis tenuifolia* and *Diplotaxis muralis.* Both are perennial being cultivated in the winter and producing new sprouts in the spring. This aspect, combined to the dehiscence of the siliqua and the large number of viable seeds, helps to spread these species as weeds. *Diplotaxis tenuifolia* is the most used for human consumption; plants are characterized by average height of 80 cm, a deep tap root, fleshy leaves, and oblong, lobed with pointed apexes.

Main differences occurred between *Eruca* and *Diplotaxis* genus in terms of plant architecture, leaf morphology, chromosomal number and phytochemical compound contents. *Eruca* species, being annuals, tends to have a higher growth rate, increased size of leaves and early flowering. These characteristics result in a high production of biomass, which make the system of cultivation different respect to the *Diplotaxis* spp. and requiring a lower seeding density and a lower number of harvests. Another trait discriminating the two genera is the larger seed dimension of the *Eruca* species (1.5 mm in length) with respect to *Diplotaxis* spp. (about 0.7 mm in length) (Padulosi and Pignone, 1996). A wider germination temperature range and greater speed of germination is also observed within the *Eruca* species, which is probably due to their annual nature, requiring greater energy of the plant to produce viable seeds (Hall *et al.,* 2015).

The consumption of rocket salad dates back since ancient time and included food and non food uses such as oil, deodorant, cosmetic and medical purposes (Hall *et al.,* 2012). Aphrodisiac properties and medical uses related to anti inflammatory and depurative effects (Padulosi and Pignone, 1996) were emphasized by ancient poets during Greek and Roman times. Nowadays leaves are eaten fresh in salads or as topping of many dishes (e.g. pizza) or cooked in soups. Several recipes provide the preparation of pureed, sauces and pesto. Other cosmetic uses concern the production of creams and lotions for body.

Rocket salad is worldwide cultivated and commercialized in many countries as mix salad packages. In Europe, the needing of prepared products ready to use as well as the major attention given to a well balanced and assorted diet, composed of a variety of health-promoting compounds, has facilitated its consumption. In central and northern markets, over half of the rocket comes from Italy and Spain, which, thanks to their geographical position and mild climatic conditions, represent the main producers. *Diplotaxis* is much more cultivated, fitting better to the needs of the farmers and being better suited to commercial utilization, thanks to the possibility to perform several harvests per cycle with yield increasing after the first harvest (Hall *et al.,* 2015).

Arugula is recommended in diets, having a very low-calorie vegetables (25 calories per 100 grams of fresh leaves) and being a very good source of vitamins and minerals (Table 2). Furthermore, it contains a range of vital compounds, with important nutraceutical and anticancer properties, which are discussed in the next paragraph.

2. Bioactive Compounds

Many studies associate a highly significant reduction in the risk of cancer as well as a tumorogenesis inhibition and hepatoprotective effects with increasing consumption of *Cruciferae* (Lynn *et al.,* 2006; Juge *et al.,* 2007; Lamy *et al.,* 2008; Alqasoumi *et al.,* 2009). Rocket contains a range of health-promoting phytochemicals including carotenoids, vitamin C, fiber, polyphenols and glucosinolates (Bennett *et al.,* 2006; Heimler *et al.,* 2007).

Table 2 - Rocket salad nutritional values for 100 g of fresh leaves (USDA Nutrient Database *)

Nutrient	Unit	Value
Energy	kcal	25
Water	g	91.71
Carbohydrate	g	3.65
Protein	g	2.58
Sugars	g	2.05
Fiber	g	1.6
Lipid	g	0.66
Vitamins		
Vitamin C	mg	15
Thiamin (Vitamin B_1)	mg	0.044
Riboflavin (Vitamin B_2)	mg	0.086
Niacin (Vitamin B_3)	mg	0.305
Pyridoxine (Vitamin B_6)	mg	0.073
Folate (Vitamin B_9), DFE [(z)]	µg	97
Vitamin A, RAE [(y)]	µg	119
Vitamin A	IU	2373
Vitamin E	mg	0.43
Vitamin K	µg	108.6
Minerals		
Calcium, Ca	mg	160
Iron, Fe	mg	1.46
Magnesium, Mg	mg	47
Phosphorus, P	mg	52
Potassium, K	mg	369
Sodium, Na	mg	27
Zinc, Zn	mg	0.47

*https://ndb.nal.usda.gov/ndb/foods/show/3569?manu=&fgcd=&ds=
[(z)] Dietary folate equivalents.
[(y)] Retinol activity equivalents.

Glucosinolates (GLSs) represent the major class of compounds in rocket and their contents in this crop have been well documented in the literature (D'Antuono *et al.,* 2008; Pasini *et al.,* 2012). When glucosinolates are exposed to myrosinase (EC 3.2.1.147, thioglucoside glucohydrolase) during tissue damage, glucose and an unstable intermediate are formed. This intermediate degrades to produce a sulfate ion, and a variety of products including isothiocyanates, nitriles and, to a lesser extent, thiocyanates, epithionitriles and oxazolidines. The relative proportion of these hydrolysis products depends on the plant species studied, on the glucosinolate itself (as side chain substitution), and reaction conditions like pH, metal ions or epithiospecifier protein (Bennett *et al.,* 2007).

Both *Eruca* and *Diplotaxis* species contain similar profiles of GLSs within the leaf tissue, the most prominent of which are glucosativin (4-mercaptobutyl-GLS), glucoerucin [4-(methylthio)butyl-GLS] and glucoraphanin [4-(methylsulfinyl)butyl-GLS]. Glucosativin and glucoerucin breakdown products are thought to contribute most to pungency and flavour in rocket (Pasini *et al.,* 2012). Numerous other GLSs have also been identified within rocket tissue, for example diglucothiobeinin [4-(b-D-glucopyranosyldisulfanyl) butyl-GLS] (Kim *et al.,* 2007), 4-hydroxyglucobrassicin (4-hydroxy-3-indolymethyl-GLS) (Cataldi *et al.,* 2007) and 4-methoxyglucobrassicin (4-methoxy-3-indolymethyl-GLS) (Kim and Ishii, 2006).

Phenolics are the most abundant antioxidants in the human diet. Considerable evidence indicates that some of the protective effects of phenols in fruits and vegetables may be due to flavonoids (Clifford and Brown, 2006). Rocket species also contain large concentrations of polyglycosylated flavonol compounds, which are known to infer numerous beneficial health effects in humans and other animals. Particularly of note are their effects on the gastrointestinal tract and in cardiovascular health (Bjorkman *et al.,* 2011; Traka and Mithen, 2011). Several studies in rocket have identified and quantified polyglycosylated flavonols, which belong to three core aglycones:

isorhamnetin, kaempferol and quercetin (Bennett *et al.,* 2006).

Pasini *et al*. (2012) studied the glucosinolate and phenolic profiles of 37 rocket salad accessions (32 *Eruca sativa* and 5 *Diplotaxis tenuifolia*) obtained by liquid chromatography-mass spectrometry. The authors isolated eleven desulpho-glucosinolates (DS-GLSs) and the glucosinolate profiles did not differ between the two species. Total DS-GLS content, expressed as sinigrin equivalents (SE) revealed a certain variability, ranging from 0.76 to 2.46 mg g^{-1} dry weight (dw) but, again, the quantitative analysis did not discriminate *Eruca* from *Diplotaxis*. Moreover, the polyphenol evaluation by HPLC-DAD-MS allowed the identification of two different classes of compounds in the two rocket salad species. Qualitative differences were observed between the polyphenol profiles at specific level: quercetin derivatives were the main phenolics of *Diplotaxis*, whereas kaempferol derivatives characterised *Eruca* samples. The contents of total flavonoids determined as rutin equivalents (RE) ranged from 4.68 to 31.39 mg g^{-1} dw. Kaempferol-3,4'-diglucoside (71.4-82.2%) and isorhamnetin-3,4'-di-glucoside (7.8-18.4%) were always isolated as first and second more abundant phenolic compounds in *Eruca* samples. No marker phenolic compounds were isolated in *Diplotaxis* samples.

Durazzo *et al*. (2013) reported significant differences in the quality of conventional and integrated cultivation practices on the nutritional properties and benefits of wild rocket [*Diplotaxis tenuifolia* (L.) DC.], while no influence on biological activity was evidenced. The authors also determined the cytotoxicity and antiproliferative activity of rocket polyphenol extract on human colon carcinoma (Caco-2) cells, evidencing a significant accumulation of cells in G1 phase and a consequent reduction in the S and G2 + M phases in response to the treatment. Regarding antioxidant properties, they found FRAP (Ferric Reducing Antioxidant Power) values ranged from 4.44±0.11 mmol kg^{-1} fresh weight (fw) to 9.92±0.46 mmol kg^{-1} fw for conventional rocket and from 4.13±0.17 mmol kg^{-1} fw to 11.02±0.45 mmol kg^{-1} fw for integrated rocket.

Villatoro-Pulido *et al*. (2013) analysing four *E. sativa* accessions reported the total content of glucosinolates ranged from 6.12 to 12.33 mg g^{-1} of dw. Glucoraphanin represented up to 52% of the total glucosinolates in leaves of one accession. Accessions showed differences in the hydrolysis of glucoraphanin to the isothiocyanate sulforaphane. No correlation between these compounds was observed, which insisted differences in the myrosinase activity within accessions. The same authors highlighted that rocket leaves had variable phenolic profiles represented by quercetin-3-glucoside, rutin, myricetin, quercetin and ferulic and p-coumaric acids. A high variability was observed for the total carotenoids ranged from 16.2 to 275 µg g^{-1} with lutein as the main carotenoid. Moreover, they found glucose was the predominant sugar, representing >70% of the total soluble carbohydrates.

Bell and collaborators (2015) used Liquid chromatography mass spectrometry (LC-MS) to obtain glucosinolate and flavonol content for 35 rocket accessions and commercial varieties. They identified 13 glucosinolates and 11 flavonol compounds; semi-quantitative methods were used to estimate concentrations of both groups of compounds. Minor glucosinolate composition was found to be different between accessions; concentrations varied significantly. According to Pasini *et al*. (2012) they confirmed flavonols differentiation between genera, with *Diplotaxis* accumulating quercetin glucosides and *Eruca* accumulating kaempferol glucosides. The authors detected several compounds in each genus that have only previously been reported in the other.

Recently, we investigated the qualitative and quantitative profiles of glucosinolates and polyphenols, highlighting flavonoid glycoside compounds (flavonols), in 39 accessions of wild and cultivated rocket (Taranto *et al.,* 2016). Seven DS-GLSs were detected in rocket leaves belonging to two chemical classes: five aliphatic compounds (glucoerucin, glucoraphanin, progoitrin, glucoalyssin, and glucosativin) and two structurally related compounds containing one intermolecular disulfide linkage, 4-(β-D-glucopyranosyldisulfanyl)butyl-GLS and dimeric 4-mercaptobutyl-GLS. The species studied significantly differed for GLS content: total average concentrations being 29.61 and 19.41 mg g^{-1} dw for *E. sativa* (21 accessions) *and D. tenuifolia* (16 accessions), respectively. Total GLS content ranged from 2.10 to 40.96 mg g^{-1} dw and from 11.61 to 26.96 mg g^{-1} dw, for *Eruca* and *Diplotaxis* accessions, respectively. Additional accessions of *D. muralis* and *Erucastrum* spp. were evaluated exhibiting an average GLS content of 17.39 and 3.63 mg g^{-1} dw, respectively.

Fifteen flavonol compounds were tentatively identified in the thirty-nine accessions studied. *Diplotaxis* accessions were characterized by nine different flavonols mainly represented by quercetin derivatives, total average content being 7.17 mg g^{-1} dw with a range from 4.91 to 8.57 mg g^{-1} dw. The most abundant flavonol compound in *Diplotaxis* was quercetin 3,4'-diglucoside-3'-(6-sinapoylglucoside). As regards *Eruca* accessions, the more abundant flavonoid group was represented by kaempferol derivatives, in agreement with a previous report (Martínez-Sánchez *et al.,* 2007). The 21 *Eruca sativa* accessions showed a flavonol total average concentration of 8.13 mg g^{-1} dw, the lowest and the highest content being 0.82 and 10.16 mg g^{-1} dw, respectively. The most abundant flavonol was kaempferol 3,4'-diglucoside.

According to previous research, isorhamnetin 3,4'-diglucoside was the only compound common to *Diplotaxis* and *Eruca* accessions studied (Martínez-Sánchez *et al.,* 2008; Pasini *et al.,* 2012; Bell *et al.,* 2015). However, some exceptions have been observed. Specific compounds mainly detected in *Eruca* such as kaempferol 3-glucoside and kaempferol 3-diglucoside-7-glucoside have been reported also in *Diplotaxis* commercial varieties (Bell *et al.,* 2015). Other compounds specific for *Diplotaxis* (i.e., quercetin 3,4'-diglucoside-3'-(6-caffeoylglucoside) and quercetin 3,4'-diglucoside-3'-(6-sinapoylglucoside) have been also identified in *Eruca* (Bell *et al.,* 2015). These inconsistencies could be related to the genetic material used. Overall the results of the analysis of glucosinolates and flavonols evidenced how the *Eruca sativa* gene pool contains potential candidates to use in breeding programs for quality.

3. Potentiality and Perspectives for Breeding

Despite the global consumption of rocket salad has increased in the recent years, little efforts have been spent by both private and public breeding programs aimed to constitute new varieties. The importance in phytochemicals has been above discussed and novel knowledge as source of resistances have been recently described (Pane *et al.,* 2017). Nowadays, constraints are mainly caused by pathogens, nitrate accumulation, early flowering and physiological disorders due to intensive culture system. Accessions of *Eruca sativa* are reported to be late-bolting (Kenigsbuch *et al.,* 2014) and to accumulate less nitrate than *Diplotaxis tenuifolia* (Cavaiuolo and Ferrante*,* 2014), being good candidates for the improvement with respect to the latter. However, several limitations for the transfer of these useful traits are linked to the failure of intergeneric crosses between *Eruca* and *Diplotaxis* due to post-zygotic barriers (Tripodi unpublished) resulting in the absence of a cost effective hybridization system available for rocket. Moreover, interspecific crosses among *Diplotaxis* species are difficult due to their different chromosomes number (Table 1).

Eruca sativa x *Brassica rapa* and *Diplotaxis tenuifolia* x *Brassica rapa* hybrids are instead possible using embryo rescue (Agnihotri *et al.,* 1990; Jeong *et al.,* 2009) and somatic hybridization (Zhang *et al.,* 2008) techniques, making the two rocket salad species a good source to use for the improvement of *Brassica rapa*. The possibility of intercross has been applied in the development of cytoplasmic male sterile (CMS) *Eruca sativa* plants transferring a male sterile cytoplasm from *Brassica oleracea* or *Brassica napus* (Merete *et al.,* 2012). Two approaches have been used: one requiring the application of embryo rescue after the first cross hybridization, subsequent chromosome doubling and backcrossing of the resulting hybrid to *Eruca* sativa, another, using protoplast fusion from cytoplasmic male sterile *Brassica*, subsequent regeneration of allogenic cells and crossing of the regenerated plant with pollen from *Eruca sativa*. The same approach has been used by Hosemans and Leviell (2012) by transferring cytoplasmic male sterility from *Raphanus sativus* to *Diplotaxis tenuifolia*. *Raphanus sativus* has been also used to transfer CMS in *Eruca sativa* (Nothangel *et al.,* 2016).

Despite these achievements, breeding activities are still carried out by means of traditional selection schemes such as mass selection or single seed descent. New possibilities may be obtained by TILLING (McCallum *et al.,* 2000) in order to select mutants for gene of interest or genome wide association approaches (GWAS) (Huang and Han, 2014) for the dissection of the genetic basis of complex traits and the development of markers for breeding assisted selection. Mutagenesis mediated by ethyl methanesulfonate (EMS) is already reported with success in *Diplotaxis tenuifolia* (Kenigsbuch *et al.,* 2014), resulting in a mutant showing late flowering and delayed postharvest senescence. These approaches successful in *Brassica* species (Stephenson *et al.,* 2010; Xu *et al.,* 2016) may be also applied in rocket salad for a better exploitation of the genetic potentiality of this crop, and furthermore, to address the challenges of the modern agriculture that demands major security and quality of foods.

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