Wild Rocket (Diplotaxis tenuifolia) Baby-Leaf

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Wild rocket (Diplotaxis tenuifolia (L.) DC) is a cruciferous perennial herb, spontaneous in the Mediterranean Basin. Here, a study on evaluating the hyperspectral response of plants to bio-based disease resistance inducers is presented.

laminarin yeast cell wall extract mixed models Trichoderma

1. Introduction

Wild rocket (*Diplotaxis tenuifolia* (L.) DC) is a cruciferous perennial herb, spontaneous in the Mediterranean Basin. In the last 25 years, Italy has become one of the major European producers of wild rocket using the species in intensified cultivation systems devoted to harvesting fresh-cut baby-leaf for the high convenience salad chain. It is sown with precision seed drills on 1.8–2.2 m width beds under polytunnels, fertigated with sprinklers and mechanically cut at complete foliar development. The harvested product quickly enters the cold chain, is minimally processed by washing, wiping, and bagging, and distributed through retail nets across several countries as ready-to-eat preparations. Packaged wild rocket meets the consumer preferences for the characteristic pungent–aromatic flavor associated with glucosinolates [1] and a few other nutraceutical properties (i.e., vitamins, antioxidants, fibers, and low calories) [2].

This makes the market very sensitive to the sustainability levels of the production process from field to shelf, conceived with a considerable reduction in the applied synthetic fungicides [3][4]. Nevertheless, this vegetable crop as well as all the other baby leaf species is susceptible to a plethora of both specific and non-specific pathogens that significantly reduce yields and impair their quality. As a consequence, the deployment of non-traditional effective control measures that include biological strategies is necessary [5].

Biologically-based disease resistance inducers may be biomimetic compounds or substances sourced from plants or microbes, or non-pathogenic microorganisms capable of eliciting the plant's own defense mechanism(s) through microbe/pathogen-associated molecular patterns and/or the recognition of host-derived damage-associated molecular patterns to enhance their innate defense response against upcoming broad-spectrum diseases to varying degrees [6][7][8]. Therefore, innovative resistance activators can then be used as biopesticides in plant protection protocols as a safer alternative to synthetic chemicals to reduce the environmental disease management footprint and stimulate plant performances [9]. However, they still need to improve their efficacy under field conditions [10].

Non-invasive technologies may be helpful to optimize the field applications of these plant-targeted protectants [11]. Plants react to exogenous application of plant resistance inducers by possibly activating many metabolic pathways involved in biochemical and mechanical defense responses including shifts of cytosolic ion content, oxidative burst, synthesis of enzymes, proteins, and other secondary metabolites related to the defense, in addition to the activation of resistance-related hormones [12]. Changes in leaf composition and plant health may be detected in the reflected electromagnetic radiation once it is captured by optoelectronic sensors such as hyperspectral ones.

In optics, reflectance measures the ability of a given surface or material to send back part of the incident light on it. In particular, a hyperspectral sensor can record the part of the electromagnetic radiation of a natural (sun) or artificial light source that is reflected by a leaf at a very fine spectral resolution in the range of wavelengths between 350–2500 nm. The spectrum is divided into three regions called, in sequence: Visible (VIS), between 350 and 750 nm; Near Infrared (NIR) until 1400 nm; and the Short-Wave Infrared (SWIR) region until 2500 nm. Each region has been associated with many parameters describing the plant status [13]. Hyperspectral data analysis represents a very effective and sustainable tool for evaluating changes induced in the plant by abiotic and biotic stresses. Recently, hyperspectral data have been adopted on a large scale in the detection of biotic stresses on plants as in the case of the sudden spread of Xylella infection on entire olive cultivations in the Mediterranean area [14][15][16]. Some vegetation indices based on reflectance data in the VIS range have been used to assess changes occurring in the plant health status through the related effect on pigments such as carotenoids, anthocyanins, and chlorophyll. However, these variations might be due to either specific or non-specific infections [17][18][19][20][21], hence further laboratory phytopathogenic analysis on the leaves remains necessary.

The transition from low reflectance values in the red to high values in the infrared spectral range is very rapid: this portion of the spectrum, called Red Edge, is more indicative of the chlorophyll content than that of water [22][23][24][25]. Moreover, it is influenced by the cell structures of leaves that poorly absorb in the NIR because of the multiple scattering of radiation by the mesophyll. As for SWIR, the overlap between information on water content and organic compounds makes data interpretation more difficult. Statistical processing [26], mathematical regressions [16], and radiative transfer models [27][28][29] have been used for this purpose.

This leads to hyperspectral data being used as indicators of possible stress in the plant, although a direct relationship between the alteration observed in the spectra and its cause has not been discovered yet.

The analysis and interpretation of spectral data are further complicated by the way in which agronomic trials are generally conducted. The purpose of the agronomic experiments is to test whether the compared treatments (in this case the use of different plant resistance inducers) have any effect on the supposed response variable. If one treatment is taken as a control (e.g., zero treatment), the experiment will consist of testing whether every other treatment has an effect compared to the control treatment. The biggest challenge in an agronomic trial is to be able to separate the intrinsic variation of the response variable from that induced by the experimental treatments. In traditional agronomic trials, this is achieved by replicating each treatment according to a well-defined experimental design. Traditional statistical methods based on the design then allow for the determination of the probability that any measured difference between treatments is due to chance (null hypothesis).

Many times, when the experiment is conducted in a confined space such as a greenhouse, or on-farm, for purely practical reasons, there is a tendency to follow a more systematic pattern, with one treatment, for example, assigned to a particular part of the field. In addition, there may be too few plots per treatment (repetitions) to assess the underlying variability, and furthermore, such variability may be correlated [30].

These experiments very often fail to meet the fundamental assumptions required by classical statistical methods. It is therefore necessary to use more complex statistical methods [31][32] that are based on a model-based statistical approach [33]. This consists in describing both the variation and the correlations between the observations of the response variable using a statistical model. In this regard, the theory of linear mixed effects models (LMM) [34][35][36] allows for the total variance to be broken down into that which is attributable to fixed effects, corresponding to the treatments, and that which is attributable to random effects. The latter are linked to the intrinsic spatial variability of the agronomic system, which cannot be described by fixed effects and can be estimated by the covariance/correlation function of residuals.

2. Discussion

The statistical results obtained are consistent with a biological interpretation, which reinforces the idea that the spectral response of the plant can be used as an effective and reliable indicator of its health. PC1 summarizes information about N content and other biochemical compounds, which to date, has come from several studies regarding the SWIR region on potato and other mapped vegetation [37][38]. As far as PC2 is concerned, the main loadings fell in the ranges more related to the LAI as confirmed by recent studies on rice and maize in both proximal and remote sensing [39][40], and to the water absorption peaks that are used to calculate new vegetation indices associated with water content in different plant species [41]. Finally, PC3 can explain the ratio Car/Chla as an indicator of plant stress [42].

It is generally recognized that the incorporation of compost into the soil increases the water available to plants [43], delaying the possible wilting associated with drought [44] and thus protecting and/or enhancing photosynthetic activity [45]. On the other hand, the lack of statistical significance of the COMPOST effect for rPC2, which was associated with plant vigor, can be explained on the basis of the reduced nutrient supply capacity shown by green composts in the presence of a large fraction of non-labile carbon, whose degradation implies the net immobilization of N [46]. In addition, compost in combination with LAM and TRI had a positive effect on the water content (PC1) and in combination with LAM and CHE on the general health of the plant (PC3). In contrast, compost in combination with CER did not produce any positive effects in terms of either water content or LAI.

However, the resistance inducers used in this study, on the basis of their specific characteristics, might be implicated in the physiological processes underlying the interpretation of the PCs. Antagonistic fungi belonging to the genus *Trichoderma* have been reported to induce a resistance response into plants through multiple hormonal signaling pathways that modulate jasmonic acid, ethylene, and salicylic acid levels toward a wide-spectrum of pathogens [47]. Their biocontrol efficacy might result in the modulation of plant growth and yield improvement [48]. Kumar and Kumar [49] reported that root colonization of *Trichoderma sp.* can induce the production of stress enzymes such as peroxidase and glutathione reductase, which may be responsible for decreasing disease incidence in *Brassica juncea*. In a different way in cabbage, *Trichoderma* treatments increased the transcript levels of genes related to photosynthesis and sucrose transport, PR proteins, chitinases, and oxidases [50]. Yeast cell-wall extract, which carries polysaccharidic and peptidic polymers and oligomers of highly variable molecular mass, acts as MAMPs in inducing defense-related events through SA signaling [51][52][53][54]. However, there is no evidence in the literature that it has an impact on the reflectance of plants. On the other hand, concerning laminarin, which is a water-soluble glucan storage polysaccharide extracted from brown algae (i.e., *Laminaria digitata* Hudson, Lamouroux), it has been shown that it can elicit defense reactions in several plant species [55] via salicylic acid and reactive oxygen species pathways [56]. This is most likely due to the association with bound β-1,3–1,6 glucosyl residues [57]. It is also worth pointing out that in this study, the LAM effect on TRI was significantly higher in all pairwise COMPOST × TREATMENT interactions relative to PC3 associated with indicating stress occurrence. Consistently, laminarin has been reported as an unconventional elicitor of plant secondary metabolites [58]. In *Arabidopsis*, this molecule increased chloroplast stability by activating the antioxidant system under stress conditions [59]. Consequently, with regard to PC3, the current hyperspectral study indicated that LAM treatment associated with the compost effect is linked to the improved plant health status.

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