Sprouts and Microgreens

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Sprouts and microgreens can be produced quickly, easily, and cost-effectively due to simple requirements for equipment and supplies, and a rapid developmental process varying from a few days (sprouts) to approximately two weeks (microgreens). This, in turn, suggests a unique opportunity for industrial scalability coupled with the prospect for consumers to independently access food with proven or purported nutritional benefits. Sprouts and microgreens have attracted tremendous interest across multiple disciplines in recent years.

plant elicitation post-harvest food safety human nutrition

1. Plant Species Scarcely Studied for Sprouting

Plant species in the *Poaceae*, *Brassicaceae,* and *Fabaceae* families are most exploited for sprouting purposes and therefore most reported by the scientific literature. For these species, research has provided a lot of information on the nutritional traits of sprouts and microgreens [1][2][3].

Other cultivated species are used for sprouting, being appreciated for several peculiar traits: vivid colors (i.e., red for red basil; green for spinach), intense smells (i.e., aromatic herbs), pleasant textures (i.e., regular for *Asteraceae*; juicy for sunflower and beet; crunchy for celery), and variable tastes (i.e., regular for *Asteraceae*; slightly sour for beet; bitter for *Cucurbitaceae*) [1][4]. In this regard, the definition and quantification of organoleptic traits represent a subject still unexplored, even for the species commonly used for sprouting, borrowing the methodology and technology used for vegetables and fruits, such as the gas chromatography-olfactometry. A first preliminary work on this has been carried out by Bianchi et al. [5] to analyze taste quality traits and volatile profile in sprouts and wheatgrass of *Triticum* spp. Another recent study compared 12 microgreens in terms of sensory attributes and visual appearance, by means of a consumer test [6]. Other grains, such as amaranth, quinoa, and buckwheat have been also studied in detail and appreciated as gluten free [7]. Several other cultivated species have been studied occasionally, for example hemp, whose sprouts are destined to become very popular [8].

This section, however, is focused on opportunities and research challenges offered by species underexplored for sprouting like voluntary species, wild relatives, ancestors, and neglected/local accessions of cultivated species, and fruit tree species. All of these would be very interesting for sprouting because they are supposed to have a higher phytochemical content compared to cultivated species [9][10][11].

Research should identify and quantify phytochemicals of these species but also investigate germination performances (% germination, mean germination time, time to reach a minimum germination threshold) and related methods for improvements. In fact, these species may show dormancy or unpredictable germination, which is a key impediment for sprouting.

1.1. Voluntary Species, Wild Relatives, Ancestors, Neglected/Local Accessions of Cultivated Species

None of these species, unlike cultivated species, were subject to breeding programs aimed at increasing yield and fruit size to the detriment of adaptability. They are characterized by high rusticity and tolerance to extreme environmental conditions that can cope with the negative effects of climate change, thereby representing potential contribution to world food security [12]. Their resilience (adaptability) is often due to biochemical mechanisms involving higher contents of secondary metabolites [13]. This also contributes to the high seedling vigor and fast vegetative growth, which warrants competitive adaptability in early growth stages [14]. For these reasons, sprouts from these species are expected to have a very high nutritional value. Some wild species were proposed and studied for micro-scale vegetables production (**Table 1**) and many other might be considered for sprouting: *Galium aparine* L. [15], *Convolvulus arvensis* L. [16], *Solanum nigrum* L. [17], and *Papaver rhoeas* L. [18]. An example of sprouts from ancestors of modern species is offered by einkorn, whose sprouts were found to be richer in phenolic substances than those of soft and durum wheat [19].

Table 1. Micro-scale vegetables from voluntary species, wild relatives, and ancient species.

Family	Species	Days after Sowing (DAS)	Secondary Metabolites	References
Amaranthaceae	*Amaranthus caudatus*–amaranth	10	PAs, total PC, FC	[20]
	Amaranthus cruentus–amaranth	4, 6, 7	Total PC, AA	[21]
	Amaranthus hypochondriacus–amaranth	2	AA	[22]
		N.S.	Carotenoids	[23]
	Chenopodium album–pigweed	48 h	AA, total PC	[24]
		48 h	Total PC	[25]
		48 h	Tocopherols	[26]
	Chenopodium quinoa–quinoa	4, 6, 7	Total PC, AA	[21]
		82 h	Total PC, AA, single phenolics	[27]
		4	Total PC, AA	[28]
		5	Total PC	[29]

Family	Species	Days after Sowing (DAS)	Secondary Metabolites	References
Apiaceae	*Chenopodium berlandieri*–huauzontle	12, 24, 36, 48, 72 h	N.R.	[30]
	Anethum graveolens–dill	8–12	Total PC, FC, AA	[31]
	Coriandrum sativum L.–coriander	N.S.	Carotenoids, total PC, single phenolics	[32]
		20	AA, carotenoids, total PC, single phenolics	[33]
Asteraceae	*Artemisia dracunculus*–tarragon	1, 3, 5, 7	N/A	[34]
	Taraxacum officinale–common dandelion	16	Anthocyanins and carotenoids	[35]
Boraginaceae	*Phacelia tanacetifolia*–phacelia	7	Total PC, AA, free and bound PAs and flavonoids	[36]
Brassicaceae	*Cichorium intybus*–chicory	12	Total PC, tocopherols, anthocyanins, carotenoids	[37]
	Diplotaxis tenuifolia–wild rocket	4, 7	Total and single GLS	[38]
		7, 21	Anthocyanins, phenolics, AA, resveratrol	[39]
Convolvulaceae	*Ipomea aquatica*–water convolvulus	8–12	Total PC, FC, AA	[31]
Malvaceae	*Corchorus olitorius* L.–jute	22	AA, carotenoids, total PC, single phenolics	[33]
Poaceae	*Coix lacryma-jobi*–adlay seed	12, 24, 36, 48, 60 h	Free, bound total PC, flavonoid, PAs and AA	[40]
	Phalaris canariensis–canary seed	24, 48, 72, 96, 120 h	Free, bound total PC, PAs and AA	[41]
	Triticum aestivum spp. spelta–spelt	5 and 12	Total PC, free and bound PAs, AA	[19]
	Triticum monococcum ssp. *monococcum*–	5 and 12	Total PC, free and	[19]

Family	Species	Days after Sowing (DAS)	Secondary Metabolites	References
	einkorn		bound PAs, AA	
		5 and 13	Total PC, FC, AA	[42]
		5 and 12	Total PC, free and bound PAs, AA	[43]
		5 and 9	Total PC, free and bound PAs, AA	[44]
	Triticum turgidum spp. *dicoccum*–emmer	5 and 12	Total PC, free and bound PAs, AA	[19]
		5 and 13	Total PC, FC, AA	[22]
		5 and 12	Total PC, free and bound PAs, AA	[42]
	Zizania latifolia–wild rice		Free, bound and total phenolics	[45]
Lamiaceae	*Salvia hispanica*–chia	7	Total PC, AA, free and bound PAs and FC	[36]
Leguminosae	*Vigna umbellata*–rice bean	6, 12, 18, 24 h	Total PC, single PAs, single flavonoids and AA	[46]
Onagraceae	*Oenothera biennis*–evening primrose	7	Total PC, AA, free and bound PAs and flavonoids	[36]
Portulacaceae [51]	*Portulaca oleracea*–common purslane [49]	1–6	N/A	[47]
		3	Free, bound soluble conjugated total PC, PAs and flavonoids	[50] [48]
	Portulaca grandiflora–moss-rose purslane	1–6	N/A [49][52]	[47]

AA = antioxidant activity; FC = flavonoid content; GLS = glucosinolates; N/A = not available; N.R. = not reported; N.S. = not specified; PAs = phenolic acids; PC = phenolic content.

...er solving ... many wild ... for weed ... well as a ... coiridoids ... ultural by- ... seeds is ... methods have been proposed for extracting phytochemicals from these kinds of seeds, while sprouting is a relatively new option, which would help producing high value food while reducing wastes. To date it has been studied only for grapeseed [53][54], pomegranate [55], olive [56], and *Citrus* species [57]. In most cases, the increase in phytochemical content and antioxidant activity observed in sprouts in relation to seeds were found to be dramatic (e.g., 30-fold for polyphenols and 90-fold for antioxidant activity in pomegranate), and much higher than in herbaceous species, thus advocating further research. However, among these species, only pomegranate showed acceptable germination rate and time, and a suitable consistency and taste to be actually used for the production of edible sprouts. In the other cases, authors realized that sprouts might better be used for extraction of food additives, cosmetics, and pharmaceuticals.

By analogy with fruit tree species, also fruit-shrub species such as blackberries, raspberries, and blueberries, can be postulated as excellent sources of sprouts, but limitations related to seed dormancy and low germination performances appear hard to overcome. However, considering the large amount of seed wastes obtained from processing, even low germination percentages could be suitable for sprouting purposes.

2. Sprouts and Human Health: Rewards and Riddles

Numerous studies have been conducted to investigate the role of sprouts as functional foods or nutraceuticals [58]. These two terms carry slightly different connotations depending on the jurisdiction of sale, regulatory considerations, and marketing strategies, but largely overlap and are frequently used interchangeably [59]. While functional foods generally imply health benefits in addition to nutritional value, nutraceuticals tend to denote a food (or a part of it) with an impact on prevention and/or treatment of a specific disease or disorder [60].

Sprouts are a good fit for nutrition research due to their wealth of phytochemical content combined with reduced antinutrient levels, and the expression of distinct cohorts of secondary metabolites that are subject to spatiotemporal modes of expression, which may largely diverge from patterns and profiles displayed in seeds and mature plants [58][61][62]. Furthermore, research offers the prospect of substantial rewards, given that products aimed at benefitting human health have progressively gained traction in recent years in response to increasing information and awareness about better eating habits and plant-forward diets, coupled with concerns associated with an aging population at risk of nutrition frailty [63]. Accordingly, markets for functional foods and nutraceuticals are growing globally at a stunning pace, accounting for revenues of US $174 billion in 2019 (forecasted to US $275 billion in 2025) for the former and US $383 billion in 2017 (forecasted to US $561 billion in 2023) for the latter (https://www.statista.com/statistics/591619/global-market-size-nutraceuticals/).

Whether sold for general health purposes or medical applications, claims about health benefits must be supported by rigorous and convincing methods of investigation prior to market launch, which may entail review and subsequent approval by governmental agencies in specific jurisdictions [64]. In this regard, nutrition science can face challenges, given the tension between scientific rigor, profitable business, and conflicting interests in relation to issues such as safety, health benefits, intellectual property, regulatory formats, marketing strategies, and financial affairs [65]. As for other complex sources of macro- and micro-nutrients, and secondary metabolites, the assessment of biological and nutraceutical effects of sprouts on human health is typically based on studies involving cellular systems representing a given phenotypic trait(s), preclinical studies in relevant animal models and, ultimately, human clinical trials. Investigational procedures, in particular cell-based studies, can be greatly facilitated by fast turnaround times to obtain samples, and the ability to enhance the content of specific metabolites using variable elicitation conditions, either abiotic (e.g., temperature, humidity, soil salinity, light intensity) or biotic (e.g., plant hormones, amino acids) [66]. On the other hand, metabolic intensity, biomechanical plasticity, and rapid physiological changes may negatively affect standardization and robustness of experimental conditions and, in turn, reproducibility and accurate interpretation of data.

Cell models generally serve as the biological systems for investigating phenotypic effects, or mechanistically identifying biologically relevant targets and related molecular networks. Within this context, sprouts provide the opportunity for implementing quick, flexible, and versatile procedures to concurrently develop metabolic variants for subsequent characterizations, comparisons to controls, and application of multivariate statistical analyses to discriminate the chemical entities exercising an active role on observed biological effects. By way of example, this rationale was applied by Ferruzza et al. [67] to show a protective effect by *Brassica oleracea* sprouts against a human model of gut inflammation developed in Caco-2 cells.

Although routinely carried out in many laboratories, tissue culture requires special attention to ensure a phenotypically relevant model for contextual interpretations of experiments, as well as robust and reproducible data. In this regard, the following are important considerations: (1) a specific cell line may exhibit significantly phenotypic variability across different laboratories, and should therefore be authenticated as a first step prior to experimentation [68][69]; (2) culture conditions and passage number should adhere to recommended protocols to avoid or at least minimize genomic and molecular alterations, while recognizing that cell populations are, to some degree, heterogeneous due to the likely presence of slow-cycling stem or progenitor cell subpopulations [70]; (3) primary cells grown on plastic (2D cultures) are rapidly subject to phenotypic changes such that they no longer reflect the biology of parental tissues [71]; and (4) cancer cell lines generally double at much higher rates (e.g., 18–24 h) than native tissues and do not represent the phenotypic multiplicity shown by the corresponding patient cohorts [72]. However, co-cultures can offer valid models to assess the effects of nutraceuticals and functional foods in that cell-cell contacts as well as paracrine or juxtacrine interactions are relevant to physiological and pathological environments, and may markedly influence sensitivity to external challenge [73]. This was clearly illustrated when quercetin and genistein, two polyphenols with proven anti-inflammatory properties, were used as positive controls to counteract the inflammatory response provoked by interleukin 1β in human pluripotent stem cell-derived endothelial cells (hPSC-EC). Notably, both polyphenols were inactive when tested in these cells, but displayed a powerful anti-inflammatory response when hPSC-EC were co-cultured with their hepatocytic counterpart hPSC-HEP that, unlike hPSC-EC, retains a metabolic capacity to breakdown quercetin and genistein into bioactive molecules [74]. Finally, it should be noted that organoids and 3D cell-based assays, despite technical challenges [75], represent the most recent advance in tissue culture to recapitulate the cellular and biological complexities of tissues and organs. Although already used for screening purposes [76], this technology is still in its infancy and, not yet employed in nutraceutical screens.

Cruciferous, grain, and legume sprouts have been tested in preclinical and human studies for their preventive and therapeutic benefits across a variety of health and pathological conditions. These investigations, extensively described and critically reviewed [3][77][78], illustrate the typical complexities of food-based trials, particularly with regard to considerations and concerns over regulatory and scientific matters, and debates about the functional and biological significance of experimental data [64][79]. In short, the same dilemma exists today as was raised by Clare M. Hasler almost 20 years ago following a 1999 guidance by the Food and Drug Administration [80]; namely, the distinction between *emerging evidence* and *strong scientific agreement* by which a product can be marketed as a dietary supplement to enhance nutrition and achieve physiological benefits, or a medical treatment to prevent or attenuate symptoms, or treat a condition. However, scientific rigor can often be questionable and not sufficiently strong to permit accurate conclusions or comparisons. For plant-derived food, including sprouts, the lack of standardized protocols for the preparation and manipulation of the natural matrix is a crucial limitation. Sprouts, in fact, have been used in animal and human studies as a whole, in the form of extracts, and juices, using different amounts and inconsistent dietary regimens within the population appraised [81][82]. Variable conditions can impact bioavailability, while elusive information about liberation, absorption, distribution, metabolism, and excretion (LADME) phases of pharmacokinetics can complicate decisions as to when and for how long a treatment should be considered [83]. For example, when ingestion of broccoli sprouts (100 g/day) was compared to the same daily amount of alfalfa sprouts (used as the placebo) in 40 (1:1 randomized) asthmatic individuals to evaluate anti-inflammatory and physiological improvements, Sudini et al. [84] used a 3-day intervention protocol that, despite having been purposely developed to mitigate variability, proved to be essentially inconclusive. Similarly, no protective effect was shown in a small-case study by Duran et al. [85], where 16 healthy individuals (1:1 randomized) followed a 3-day diet including 200 g/day of either broccoli or alfalfa sprouts (200 g/day in both cases) prior to exposure to ozone-induced neutrophilic airway inflammation. Conversely, Brown et al. [86] showed that a sulforaphane-enriched extract from broccoli sprouts taken for 14 days by 45 moderately asthmatic patients beneficially impacted the methacholine bronchoconstriction challenge in 60% of cases, with 20% of patients negatively affected and the remaining 20% unresponsive. Notably, while the study from Duran did not identify any change in the expression of NRF2 (Nuclear factor erythroid 2-related factor 2)-dependent genes (known to be induced by sulforaphane), Brown et al. [86] found instead that activation of the NRF2 pathway correlated with bronchoprotective benefits. Overall, these studies show that divergence of key experimental factors, such as quality and quantity of the material used, time of intervention, size of population tested, and endpoint measurements, can lead to inconsistent conclusions, further exacerbated by population heterogeneities [87], as well as bi-phasic or hormetic responses commonly raised by bioactive agents including phytochemicals [88]. Landberg et al. [89], for example, advanced the importance of a methodological approach to stratify clinical trial participants based on the integration of different parameters such as genetic (polymorphisms), phenotypic (age, gender, body mass index (BMI), and the gut microbiome) and ADME profiles to define metabolic representations, namely metabotypes, to ultimately strengthen the association between the effect of plant-based food or any of its (semi)purified components and a specific group of individuals, thus, implementing what it could be referred to as *personalized or precision nutrition*.

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