*agronomy*

*Review*

# Seed Priming Applied to Onion-Like Crops: State of the Art and Open Questions

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**Abstract:** Cultivation of onion and onion-like crops has become a challenge in the context of climate change and innovative solutions are needed to support breeders as well as farmers, starting from the crucial issues of seed quality. Seed priming is routinely used to enhance germination performance and seedling resilience. Although common for radish, tomato, carrot or head cabbage seeds, the technique has been less popular for onion-like crops. This review provides a state-of-the-art picture of the scientific and technological advances that have so far contributed to enhance seed germination and vigour in onion-like crops. A brief description of the different types of priming approaches is provided whereas attention is focused on the following: (i) the impact of seed priming in terms of improved germination, seedling development and resilience to environmental stresses; (ii) the concept of seed quality hallmarks translated from model plants to the genus *Allium*; (iii) the drawbacks that currently impair the fully exploitation of seed priming in this specific sector of the agrifood chain.

**Keywords:** *Allium*; seed germination; seed priming; seed quality hallmarks; plant field stand

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## 1. Introduction

The genus *Allium* (monocot, botanical family Alliaceae, previously named as Amaryllidaceae) covers approximately 850 species that are widely cultivated for food and medicinal purposes. They represent a valuable source of micronutrients (minerals and vitamins) and bioactive molecules (e.g., organosulfur compounds, polyphenols, saponins, fructans, fructo-oligosaccharides) found in their extracts and essential oils. The *Allium*-derived products are known for their antioxidant, antibacterial, anti-inflammatory, antiproliferative and anticancer activities, and they are also useful for food biopreservation and nanoformulates [1]. These hardy perennial plants, characterized by true or vestigial bulbs associated with rhizomes, account for a significant part of the horticultural market, in terms of both fresh-eating and processed bulbs. The group includes onion (*Allium cepa* L.), garlic (*Allium sativum* L.), wild leek (*Allium ampeloprasum* L.), chive (*Allium schoenoprasum* L.), shallot (*Allium ascalonicum* L.), Welsh onion (*Allium fistulosum* L.), Chinese chive (*Allium tuberosum* Rottl. ex Spr.), leek (*Allium porrum* L.) and Jiaotou (*Allium chinense* G. Don).

In recent years, there has been a clear tendency amongst the leading producers of onion-like crops in the world to increase the supply of both fresh and processed onions on the market. According to FAO [2], the world production in 2020 was estimated 115.24 million tons and 4.90 million tons for dry (excluding dehydrated) and green onions and shallots, respectively. The total world production of garlic in 2020 was 28.05 million tons, with China accounting for up to 74% of the global production (20.71 million tons) [3]. The top producers of dry onions in 2020 include India (26.31 million t), China (23.34 million t), the U.S. (3.82 million t), Egypt (3.15 million t), Turkey (2.28 million t), Pakistan (2.12 million t), Iran (2.06 million t), Russia (1.73 million t) and Brazil (1.49 million t) [2].

All the *Allium* species, except shallot and garlic, are commercially propagated through seeds. Their seeds have thin coat (testa), do not go through a dormancy period and lose their germination capacity quickly, i.e., within 6–12 months [4–6]. As onion-like crops seed production in the EU lies under the regulation of the standard category, no data are currently available about their domestic seed market turnover [5]. In China, the country with the biggest production of vegetables in the world, although the importance of onion-like crops is smaller than tomato, Chinese cabbage or early potato, these crops still represent an important group [6–8]. It is clear from the available data that onion, out of all onion-like crops, is the most relevant crop in the world production. The same tendency is observed in research, since onion has been prevalently investigated as the main target for basic and applied studies.

High-quality seeds are essential to support crop yields. As for onion-like products, there is a strong market demand for a significantly higher germinability ensuring high propagation and robust plant emergence. According to FAO, quality declared seed systems require the minimum germination of 70% for onion seeds [9]. Unfortunately, onion seeds exhibit poor performance due to attributes like limited longevity and storability, which ultimately result in rapid loss of viability [10]. Onion seeds are small in size and their seed establishment is poor. Low-quality seeds result in low and asynchronous germination and high numbers of abnormal seedlings under stress conditions in early spring planting [11]. Onion seed quality is dependent on environmental parameters occurring during plant growth and seed development, seed location on the mother plant, harvest period and techniques, storage conditions and pre-sowing treatments. Viability and vigour during storage are extremely variable, depending on species/cultivars and even seed lots [12,13]. In this context, seed priming is regarded as a promising option to rescue viability in low-quality onion seeds.

## 2. Seed Priming: Basic Principles and Technology

Seed priming was first proposed by Heydecker and co-workers [14,15]. The concept of 'priming of seeds' was introduced in 1977, at the Symposium on Seed Problems in Horticulture (Sutton Bonington, UK) and published as a Conference Paper [14]. Heydecker and co-workers successfully applied the osmotic agent polyethylene glycol (PEG) to onion and carrot seeds to increase both the rate and uniformity of germination [14]. In 1977, the International Seed Testing Association (ISTA) defined 'seed vigour' as the combined expression of those seed properties that determine the activity level and behaviour of a seed or seed lot during germination and emergence. In the following years, methods to improve seed vigour have been extensively studied, and the concept of 'seed priming' has gradually been adopted by the international seed biology community. The seed priming technicques have been continuously developed, improved and commercialized as an effective method to enhance seed vigour [15–20].

The process of seed priming requires the controlled imbibition of dry seeds in order to (i) allow the resumption of metabolic activities (the so-called 'pre-germinative metabolism'), (ii) bring seeds into an advanced physiological stage and (iii) boost some key molecular events, namely the antioxidant and DNA repair responses. The latter contributes to preserve genome integrity, an essential requisite for successful germination [21]. The controlled seed imbibition, performed using only water or by adding a range of different priming agents, is then stopped before radicle protrusion occurs, otherwise the seed will lose desiccation tolerance. In this way, primed seeds will survive the subsequent dehydration or 'dry-back' that will bring them to standard moisture content for storage or to be sown in the field [16]. Seed priming-based methods include different types of treatments: hydropriming, osmopriming, drum priming, solid matrix priming, biopriming, hormonal priming, nanopriming, physical priming and hybrid priming. For each approach, a brief description is provided whereas the related advantages and limitations are listed in Figure 1.

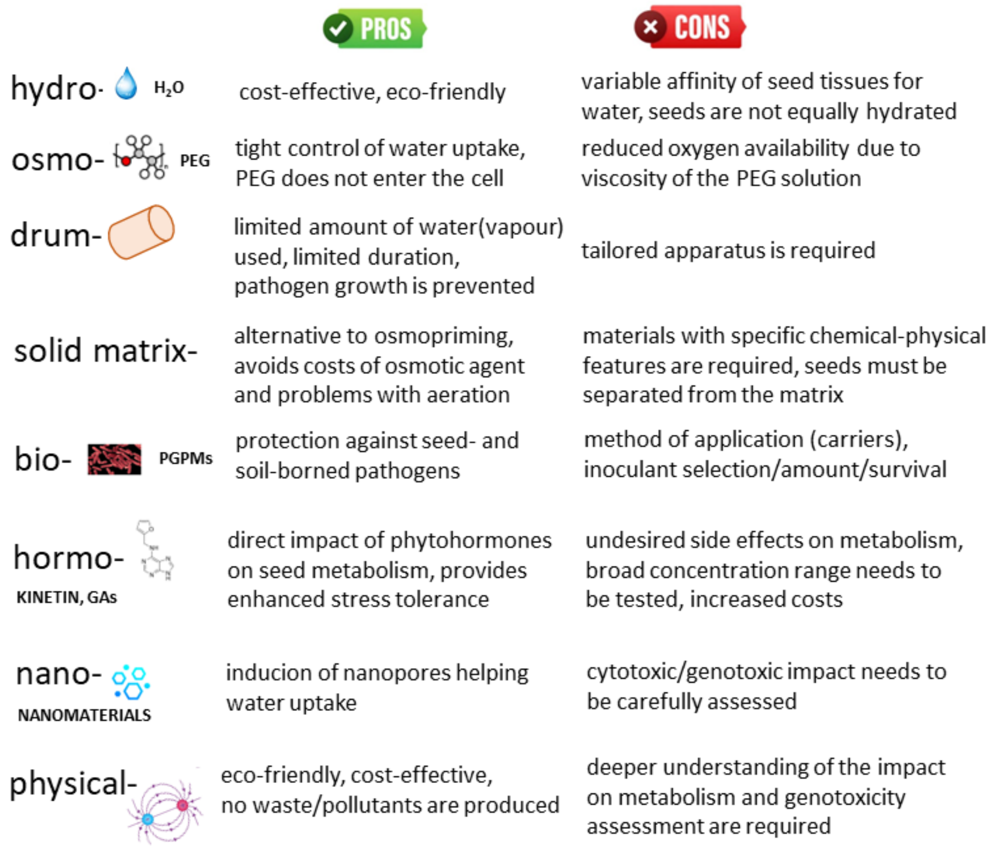


**Figure 1.** Advantages and limitations of the main seed priming techniques.

*Hydropriming.* Seeds are directly soaked in water and imbibition is carried out under controlled conditions of time, temperature and oxygen supply. This cost-effective, eco-friendly priming approach shows some limitations related to the treated species/cultivars. Indeed, each genotype can differ in terms of time required for soaking, and such threshold should be carefully assessed [22].

*Osmopriming*. Seeds are immersed in a ventilated, low-water potential solution. The osmotic pressure of water is adjusted by controlling the concentration (water potential) of the osmotic solution, thereby controlling the amount of water absorbed by the seeds. Commonly used solutions include osmotic agents as PEG, sodium poly-propionate (SPP), polyvinyl alcohol (PVA) and salts (e.g., $KNO_3$, NaCl) [23].

*Drum priming*. A specialized roller-type equipment is used for this method. The amount of supplied water, treating time and temperature are precisely controlled by computer to let the seeds absorb water to a desired level. The treated seeds are then placed in a container for a defined period of time. Next, they are either sown on the field or dried to their initial moisture level. This method is precise in control and convenient for large-scale commercial processing [24].

*Solid matrix priming*. Seeds are mixed with solid substrate and water in a specific ratio in order to simulate seed imbibition in soil [25]. The seeds absorb water through the substrate to reach the balance level. The most commonly used substrates are vermiculite, sand, perlite, shale and synthetic calcium carbonate. The solid matrix material needs to be separated without damaging the seeds [25].

*Biopriming*. Beneficial microorganisms are added as seed protectants during seed hydration. They can be encapsulated on the seeds by film-forming agents or directly added to the substrate. This approach relies in most cases on the use of microorganisms belonging to the genera *Pseudomonas*, *Enterobacter*, *Bacillus* and *Trichoderma* [26].

*Hormonal priming*. Phytohormones, including gibberellins, abscisic acid, cytokinins, salicylic acid and jasmonic acid, are used as priming agents. Hormonal priming has beneficial effects since it promotes seed dormancy release and improves seedling tolerance to stress [27].

*Nanopriming*. This method, recently developed, is based on the use of nanoparticles (NPs) as priming agents. A wide range of NPs has been so far used for seed treatments, including FeNPs, AgNPs, $SiO_2$NPs, CuONPs and ZnONPs. Plant-based NPs are also available, as a result of sustainable processes that produce safe nanomaterials for agricultural applications [28,29].

*Physical priming*. Seeds are treated with physical techniques such as magnetic fields, ultrasonic waves, ultraviolet rays, x-rays, $\gamma$-rays and microwaves. Their main advantages are that these are low-cost, easy-to-manage techniques, and the fact that they do not release pollutants [30].

## 3. Seed Priming Applied to the Genus *Allium*

### *3.1. Onion*

Onion seeds have been tested with different priming protocols, and below a brief review of the most effective treatments is reported. In 1988, Ellis and Butcher [31] tested osmopriming on different seed lots of the White Lisbon and Senshy Semi Globe Yellow cultivars, providing for the first time evidence of the genetic and environmental factors contributing to the seed response to the treatment. Osmopriming reduced the thermal time for germination at both sub- and supra-optimal temperatures for the tested onion cultivars; however, the observed effect was seed lot-dependent [32]. This study highlighted the usefulness of thermal time models to determine key requirements for onion seed germination as well as the ability of osmopriming to positively target the seed response in terms of thermal and temporal requirements for optimal germination. This approach can be particularly informative, considering that nowadays it is essential to understand the seed performance under changing climate conditions [32]. Seeds of onion cultivars Bronze Wonder, Challenger, Big Mac and White Keeper were successfully osmoprimed, resulting in improved seedling emergence, particularly in field spring plantings [33]. The response of osmoprimed onion seeds in the field experiments was affected as the soil temperature increased, underlining issues related to high genotype- and seed lot- dependent variability [33]. Large-scale osmopriming applied to onion seeds was achieved through the careful management of oxygenation. The treatment performed in the presence of oxygen-enriched air (up to 75% $O_2$) resulted in increased germination percentage [34]. Cellular respiration and energy metabolism are triggered upon imbibition and supported by oxygen transfer across the seed coat. Since oxygen supply can be reduced in presence of water or osmotic agents, thus compromising the metabolic activities, exposure to oxygen-enriched air is used to boost these key processes and improve seed quality [35]. Priming of onion seeds with trehalose or raffinose solutions accelerated germination speed and uniformity, also under abiotic stress conditions, e.g., salinity (100 mM NaCl), osmotic stress (10% PEG) and low temperature (15 °C) [36]. Germination rates of primed onion seed increased up to 30–50% whereas untreated seeds showed a poor performance (2%) [36]. Priming with trehalose provided protection against chilling in rice (*Oryza sativa* L.) seeds [37]. Trehalose-primed rice seeds tested at low temperature (15 °C) showed reduced malondialdehyde levels (hallmark of lipid peroxidation), upregulation of *OsTPP* (*Trehalose-6-Phosphate Phosphatase*) and antioxidant (peroxidase, catalase, ascorbate peroxidase) genes, as well as increased levels of proline and soluble sugars (required for osmotic balance) [37]. It is possible that these highly conserved metabolic pathways and protection mechanisms contribute to the beneficial effects of trehalose-mediated priming reported in onion seeds. Nutripriming with 0.5% $ZnSO_4$ and hormonal priming with 0.2% methionine applied to onion seeds (cultivar CO (On) 5) promoted salt stress tolerance as evidenced by increased germination percentage, dry matter production and vigour index [38]. Nutriprimed seeds showed enhanced cell division and elongation rate in the root meristem, as revealed by scanning electron microscopy, and they were able to withstand low moisture conditions (20% water holding capacity) and salt stress (up to 0.75% NaCl) [38]. The added value of zinc as priming agent relies on its role as an essential trace element, in the control of several physiological, biochemical and molecular processes among which is germination, with positive impact on plant growth, crop yield and nutritional quality [39]. Methionine is a precursor of the phytohormone ethylene known to promote germination and this might represent the physiological process underlying the response of onion seeds [40]. Bulb diameter and weight that contribute to onion bulb yield were significantly improved by hormonal priming with 100 ppm $GA_3$ and halopriming with 3% $KNO_3$ in the cultivar Agrifound Dark Red [41]. $GA_3$ promotes seed dormancy release, germination, cell and plant growth also under salinity and drought stress by triggering the antioxidant defense and proline accumulation [42,43]. Caseiro and co-workers [44] compared the effect of osmopriming with an aerated PEG 8000 solution (−0.5 MPa, −0.1 MPa), hydropriming and drum priming on the germination speed and percentage in six lots of onion seeds differing in quality. Although hydropriming and osmopriming provided the best response, the most cost-effective technique was drum priming [44]. Hormonal priming with 50 μM salycilic acid was able to increase germination and growth performance of the cultivar IPA 11 under water and salt stress conditions [45]. Onion seedlings developed from primed seeds showed an enhanced capacity of osmotic adjustment under severe PEG-induced water stress (−0.2 MPa) and NaCl-induced salt stress (−0.4 MPa) by increasing proline and soluble sugars contents [45].

The study by Dearman and co-workers [46] highlighted that osmopriming with PEG was able to mitigate the impact of ageing in stored onion seeds whereas the treatment performed after ageing did not improve seed viability. The deleterious effects of ageing were ameliorated in onion seeds (cultivar Punjab Red-48) subjected to osmopriming with 25% PEG. Increased levels of antioxidants, e.g., tocopherols and ascorbic acid, as well as catalase and peroxidase antioxidant activities were observed in treated seeds [47]. The impact of different seed priming treatments (hydropriming plus dry coating with 2 g/kg Thiram; hormonal priming with 50 ppm $GA_3$; osmopriming with 0.5% KNO3 and 0.5% KH2PO4, respectively; biopriming with *Azotobacter*) was assessed on naturally aged onion seeds (cultivars Hisar-2, Hisar Onion-3, and Hisar Onion-4) [48]. $GA_3$ was found to be best priming treatment for improving seed quality of the tested onion cultivars, followed by biopriming, hydropriming and coating. Hormonal priming and biopriming were proved to increase seed viability under one-year storage [48]. Muruli and co-workers [49] reported that in aged seeds of the Arka Kalyan cultivar germination was improved by osmopriming with PEG (−1.5 MPa) whereas fresh seeds were more responsive to $GA_3$-mediated hormonal priming. These authors underline the different effects of the tested priming agents observed in fresh and aged onion seeds, respectively, and the need to expand the range of protocols to speed the search for the most cost-effective solutions [49]. Onion has become a useful component of the recently developed green nanotechnology for sustainable precision agriculture [50]. Silver and gold nanoparticles (AgNPs, AuNPs) synthesized from onion extract were delivered to aged onion seeds. Instrumental neutron activation analysis and gas chromatography-mass spectrometry showed that nanoparticles were internalized, resulting in enhanced germination performance under both greenhouse and field conditions, and associated with increased peroxidase activity [50].

The use of magnetic field as a simple, inexpensive and non-invasive physical method to stimulate germination in crop plants has been reported [30]. Physical vigorization treatment has been applied, e.g., the effects of static magnetic field on Yellow Granex PRR hybrid onion seed germination and seedling growth were described [51]. When seeds were exposed to static magnetic fields of 10 and 21 mT (milliTesla) for increasing time (0.5, 3, 6, 12 and 24 h), no significant differences in germination, dry or fresh weight were observed. However, an increase in the length of seedlings developed from magnetoprimed onion seeds was reported [51]. Commercial seed lots of cultivars Octavia and Eureka subjected to low frequency magnetic field (LFMF) showed improved germination [52,53]. Imbibed seeds were exposed to LFMF (20 mT) for 10, 30 and 60 min, tested for germination performance, and the resulting bulbs were analysed for root length, maximal diameters, dry matter and quercetin content [52,53]. Both cultivars showed enhanced germination in response to LFMF treatment of 60 min. 'Octavia' onion seeds produced bulbs with increased root length and quercetin content. The latter is a useful character for onion breeders since it influences the taste and the health-promoting potential of the bulb [54].

Nowadays, seed priming applied to onion is an expanding technique that benefits from the more advanced knowledge acquired in other model and crop plants. Table 1 summarizes the seed priming treatments currently developed for onion seeds. The limited body of literature so far available on the impact and limitations of seed priming in onion is expected to increase, especially when the efforts of basic and applied research will be combined and innovative tools (e.g., omics) will be used to explore the dynamics of the pre-germinative metabolism.

**Table 1.** Summary of the seed priming methods applied to onion seeds.

| Cultivar | Priming Method (*) | Reference |
|---|---|---|
| Granex 33 | hydropriming: water, 15 °C, 96 h | Caseiro et al. 2004 [44] |
| Granex 33 | drum priming: water, 15 °C/25 °C | Caseiro et al. 2004 [44] |
| White Lisbon; Rijnsburger Wijbo | osmopriming: 34.2% PEG 6000, 15 °C, 10 d | Dearman et al. 1986 [46] |
| White Lisbon; Senshy Semi Globe Yelllow | osmopriming: −1.4 MPa, 34.2% PEG 6000, 20 °C, 7 d | Ellis & Butcher 1988 [31] |
| Rijnsburger Robusta | osmopriming: −1.5 MPa, 34.2% PEG 6000, EA, 15 °C, 14 d | Bujalski & Nienow 1991 [34] |
| Bronze Wonder; Challenger; Big Mac; White Keeper | osmopriming: 30% PEG 8000, 10 °C, 7 d | Murray et al. 1992 [33] |
| Granex 33 | osmopriming: 30% PEG 8000, 15 °C, 24/48 h | Caseiro et al. 2004 [44] |
| Arka Kalyan | osmopriming: −1.5 MPa, PEG 6000, 25 °C, 24 h; osmopriming: 3% $KNO_3$, 25 °C, 24 h | Muruli et al. 2016 [49] |
| Punjab Red-48 | osmopriming: 25% PEG 8000, 20 °C, 5 d | Basra et al. 1994 [47] |
| Hisar-2; Hisar Onion-3; Hisar Onion-4 | osmopriming: 0.5% $KNO_3$, 16 h; osmopriming: 0.5% $KH_2PO_4$,16 h | Brar et al. 2019 [48] |
| Furanui; Tsukihikari; Early Grove | osmopriming: 0.4 M trehalose /raffinose, 15 °C, 4−6 d | Horita & Saruyama 2006 [36] |
| CO (On) 5 | nutripriming: 0.5% $ZnSO_4$, r.t., 10 h | Saranya et al. 2017 [38] |
| Agrifound Dark Red | halopriming: 3% $KNO_3$, r.t., 12 h | Thejeshwini et al. 2019 [41] |
| Arka Kalyan | hormonal priming: 50 ppm $GA_3$, 25 °C, 24 h | Muruli et al. 2016 [49] |
| CO (On) 5 | hormonal priming: 0.2% methionine, r.t, 8 h | Saranya et al. 2017 [38] |
| Agrifound Dark Red | hormonal priming: 100 ppm $GA_3$, r.t., 6 h | Thejeshwini et al. 2019 [41] |
| Hisar-2; Hisar Onion-3; Hisar Onion-4 | hormonal priming: 50 ppm $GA_3$, 16 h | Brar et al. 2019 [48] |
| IPA 11 | hormonal priming: 50 µM SA, 25 °C, 24 h | Da Silva et al. 2019 [45] |
| Hisar-2; Hisar Onion-3; Hisar Onion-4 | biopriming: *Azotobacter* strain HT−57, 16 h | Brar et al. 2019 [48] |
| 1015Y Texas Super-sweet onion (variety Legend) | nanopriming: 31.3 ppm AgNPs, r.t., 12 h; nanopriming: 5.4 ppm AuNPs, r.t., 12 h | Acharya et al. 2019 [50] |

(*) Abbreviation used in the description of the priming method: PEG—polyethylene glycol; d—day; h—hour; MPa—megaPascal; EA—enriched air; ppm—parts per million; AgNPs—silver nanoparticles; AuNPs—gold nanoparticles; r.t.—room temperature.

### 3.2. Leek

Early studies on primed leek (*Allium porrum* L.) seeds contributed to better understand some molecular events associated with germination [55,56]. Davidson and Bray [55] reported that osmopriming with PEG (−1.0 MPa) improved germination performance of a high-quality seed lot of the cultivar Verina. Several polypeptides were specifically synthesized in the leek endosperm tissue during priming and subsequently along germination [55]. Clarke and James [56] described enhanced levels of RNA species in embryos and other tissues of primed leek seeds. In another early study [57], different seed lots of the leek cultivar Winterreuzen primed with PEG showed improved germination rate and uniformity, under both field and greenhouse conditions. These authors also highlighted the crucial impact of dry-back that may delay leek seed germination and emergence [57]. Leek seeds are characterised by low emergence rate that causes poor stands and, based on early investigations, priming appeared as a promising tool to overcome such drawbacks at low temperature (15 °C). Since seed germination is impaired at temperatures higher than 25 °C, Parera and Cantliff [58] addressed the issue of leek plant establishment under high temperature. Seeds of the leek cultivar Verina were primed in aerated solutions (−1.5 MPa) of D-mannitol, PEG, and $KNO_3$, resulting into early emergence at high temperature and improved stand uniformity for container transplant production, usually susceptible to heat stress [58]. Osmopriming was reported to improve germinability of leek seeds at suboptimal temperatures and under hypoxia (low oxygen partial pressure) [59]. Crop plants can be subjected to hypoxia caused by transient flooding whereas oxygen deficiency can occur in poorly drained soils, severely affecting plant establishment, growth and productivity. Sensitivity to oxygen deficiency and mechanisms for metabolic adaptation to low oxygen in germinating seeds is dependent on several molecular factors, e.g., ROS levels and the balanced activity of ROS producing and ROS scavenging enzymes [60]. These issues need to be urgently explored in onion and onion-like crops, considering the impact of waterlogging conditions caused by heavy rainfall in sensitive regions [61]. Different priming agents (KCl, $KH_2PO_4$, $CaCl_2$ and PEG 8000) were tested on seeds of the leek cultivars Lungo della Riviera and Monstrueux de Carentan [62]. Germination percentage of the cultivar Lungo della Riviera was improved by PEG and KCl, whereas $CaCl_2$ improved germination of the cultivar Monstrueux de Carentan [62]. The hydrotime model was used to test the effectiveness of the different priming agents as well as the intraspecific variability in germination percentage and speed under water stress [62]. Priming with L-tryptophan (125, 250, and 375 ppm) and melatonin (5, 10, 25 uM) was applied to leek seeds and germination was tested under optimum temperature (21 °C), chilling stress (7 °C), and hot temperature (35 °C). Treatment with 125 ppm L-tryptophan and 5 µM melatonin, respectively, significantly improved germination under chilling stress and seedling resilience [63]. Table 2 summarizes the seed priming treatments currently developed for onion seeds.

**Table 2.** Summary of the priming methods applied to leek and Welsh onion seeds.

| Species/Cultivar | Priming Method (*) | Reference |
|---|---|---|
| Leek/Winterreuzen | osmopriming: −15 bars, 34.2% PEG, 15 °C, 7/14/21 d | Brocklehurst et al. 1984 [57] |
| Leek/Verina | osmopriming: PEG 8000, 15 °C, 14 d | Clarke & James 1991 [56] |
| Leek/Verina | osmopriming: −1.0 MPa, PEG 6000, 15 °C, 14 d | Davison & Bray 1991 [55] |
| Leek/Verina | osmopriming: −1.5 MPa, PEG 8000/D−mannitol/$KNO_3$, 15 °C, 10 d | Parera & Cantliff 1992 [58] |
| Leek/Premier | osmopriming: −15 bars, PEG 6000, 15 °C, 1−14 d | Corbineau et al. 1994 [59] |
| Leek/Lungo della riviera; Monstrueux de Carentan | osmopriming: −1.8 MPa, PEG 8000/KCl/$CaCl_2$/$K_2HPO_4$, 20 °C, 96 h | Romano & Bravi 2021 [62] |
| Welsh onion/Zhangqiu | osmopriming: 2% $KNO_3$, 25 °C, 24 h | Dong et al. 2014 [64] |
| Leek/Inegol-92 | hormonal priming: 125 ppm L−tryptophan, 20 °C, 24 h, dark; hormonal priming: 5 µM melatonin, 20 °C, 24 h, dark | Hanci et al. 2019 [63] |

(*) Abbreviation used in the description of the priming method: PEG—polyethylene glycol; d—day; h—hour; MPa—megaPascal; ppm—parts per million.

While onion varieties require specific temperature and day-length conditions, leek varieties are characterised by a larger adaptability. This is due to the fact that leeks do not produce bulbs and do not need a rest period. Leeks can be harvested over prolonged periods and own enhanced cold tolerance, compared to onions. Such adaptability as well as the availability of effective priming protocols might contribute to better withstand climate changes in agriculture and food systems.

### 3.3. Welsh Onion

The Welsh onion (*A. fistulosum* L.) (also called stone leek, Chinese onion, Chinese spring onion and Japanese bunching onion), less known than onion, is commercially produced in Japan, China and South Korea [4]. Dong and co-workers [64] tested the impact of priming with $KNO_3$ on the germination performance of cultivar Zhangqiu. Germination rate and vigour index of Welsh onion were significantly improved. Membrane integrity, assessed by measuring soluble sugar content and electrical conductivity of the seed leachates, was preserved. The primed seeds also showed reduced hydrogen peroxide and malondialdehyde levels as well as increased activity of superoxide dismutase and catalase [64]. The current limited state of the art (Table 2) focused on Welsh onion highlights the need for robust research efforts addressing the issues of seed quality, including seed performance under storage [65].

### 3.4. Seed Priming in Onion and Onion-Like Crops: Major Limitations and Lessons Learned

When summarizing results of the experimental activity carried out to assess the impact of seed priming in onion and onion-like crops, PEG-based osmopriming proved to be the main method used (Tables 1 and 2), although it is evident that the efforts so far made did not converge into a unique, universal protocol. Rather, looking at the details of the different treatments, there are parameters (PEG molecular weight and concentration, temperature, time, presence/absence of aeration or even enriched air) that needed to be adjusted, depending on the genotype and/or the seed lot. Indeed, the main advantages of this method (simple procedure combined with low-cost application) must cope with a major limitation of the priming technology, which is still empirical and not always reproducible. As a consequence, the work of seed company operators is delayed and the search for innovative solutions has become a priority. Given these problematic issues, it is also evident that poor attention has been so far dedicated to seed priming for onion and onion-like crops. A survey of the scientific literature carried out using PubMed® highlights how research in this field is still underrepresented (Table 3). Differently from major cereals (rice, wheat, maize), the reports dealing with horticultural crops are dominated by tomato and soybean whereas onion and leek stand for the scanty number of dedicated papers.

**Table 3.** Timeline and total number of published papers dedicated to seed priming as well as occurrence of reports focused on seed priming applied to main crop plants. Information retrieved from PubMed® (https://pubmed.ncbi.nlm.nih.gov/; accessed on 5 January 2023).

| Key Words | Timeline | Publications (N°) |
|---|---|---|
| seed priming | 1970–2023 | 1.151 |
| seed priming, rice | 1995–2023 | 148 |
| seed priming, wheat | 1980–2023 | 129 |
| seed priming, maize | 1970–2023 | 77 |
| seed priming, tomato | 1995–2023 | 52 |
| seed priming, soybean | 1985–2023 | 43 |
| seed priming, pepper | 2000–2023 | 7 |
| seed priming, onion | 2010–2023 | 7 |
| seed priming, leek | 2010–2023 | 4 |
| seed priming, eggplant | 2017–2023 | 4 |

Such a scenario might sound as an alarm bell to the operators (breeders, seed technologists, agronomists, researchers) acting at different levels of the onion and onion-like production chain. Financial and cultural efforts shoud be boosted to speed up basic and applied research in this field, taking advantage of the information already available on the dynamics of the pre-germinative metabolism in the most studied model and crop plants.

## 4. Molecular Hallmarks: From Model Plants to *Allium* Crops

Despite the increasing focus on seed priming techniques as tools to overcome the issues of seed quality in the context of global climate change [16,17,20], there are still several drawbacks that delay many successful applications on cultivated plants, including onion-like crops [66]. The effectiveness of priming protocols relies on crucial factors that are genotype- and seed lot-dependent [16–19]. Prolonged imbibition can result in overpriming or premature radicle protrusion occurring during the treatment. Overprimed seeds lose desiccation tolerance and cannot survive the dry-back step. Deleterious effects on seed viability are also observed if the desiccation rate during dry-back is not properly optimized [16–19,67,68]. Since the rehydration-dehydration timing is currently optimized empirically, a deeper understanding of the molecular dynamics ruling desiccation loss and gain is required [69,70]. According to Dorone and co-workers [71], the *Arabidopsis* seed proteome is enriched in intrinsically disordered proteins, including prion-like proteins involved in protein phase separation. This physical process, well known in polymer chemistry, allows the compartmentalisation of biomolecules into membraneless supramolecular assemblies [72]. Dorone and co-workers [71] identified the novel prion-like protein FLOE1 acting as a putative plant water potential sensor in the *Arabidopsis* seed. The protein shows reversible hydration-dependent phase separation and regulates seed germination under drought stress, and such role could be exploited in biotech and agronomic research programmes to boost seed quality and stress resilience in crop plants. The different processes involving DNA (recombination, repair, transcription) are tightly dependent on the chromatin status that is modulated through epigenetic modifications (DNA methylation, histone modifications). Chromatin remodeling, another key player in the control of seed germination, is still poorly investigated in this context [73]. Genes encoding proteins involved in chromatin remodeling might represent useful indicators of the germination performance. Yang and co-workers [74] demonstrated that chromatin remodeling is required to silence the *AtNCED6* (*9-CIS-EPOXYCAROTENOID DIOXYGENASE 6*) gene that encodes a rate-limiting enzyme in ABA biosynthesis. Such mechanism requires the synergic action of RNA-binding protein RZ-1 and polycomb repressive complex 2 (PRC2) in order to promote H3 deacetylation and suppress H3K4me3 accumulation. Thus, progressive chromatin condensation mediated by these actors results into repression of ABA biosynthesis genes and triggers germination [74].

Seed technologists seek for innovative tools that can support them in the early screening of seed quality and contribute to develop smart and sustainable procedures in this sector. Molecular hallmarks that allow to monitor the seed response to priming using easy-to-manage assays represent an option currently explored by researchers in crop species, e.g., legumes [69,70] and Solanaceae [71,72]. The main processes, namely antioxidant response and DNA damage response, are regarded as sources of putative hallmarks of seed quality [75–82]. ROS detection and quantification is used to assess the seed response to priming, along the rehydration and dry-back steps [70,78,79] whereas the expression profiles of antioxidant and DNA repair genes measured through quantitative realtime PCR provide information about the way seeds manage oxidative and genotoxic damage during treatments [19, 70–80]. The knowledge so far accumulated in model species should be translated to crop plants, including the onion-like crops. It is worth noting that Ashraf and Bray [81] first reported the experimental evidence of DNA repair in osmoprimed leek seeds in 1993. Due to the highly conserved features of the DNA damage response in plants, such translational research approach will be facilitated. In this context, one of the main weakness points relates to the scanty use of omics tools to unravel the molecular pathways underlying plant growth and stress responses in *Allium* species [82]. Despite its economic and medicinal importance, the first genome assembly of onion was reported only recently, a result possibly delayed due to the large genome size [83]. Omics high throughput technologies, genomics, transcriptomics, proteomics, metabolomics and metagenomics can provide huge datasets and knowledge essential to open new routes for the genetic improvement of onion and onion-like crops. A few reports describe transcriptome analysis in onion plants to decipher the drought stress tolerance response [84] or phytohormone-triggered developmental processes [85], as well as the use of proteomics to address the issue of bulb dormancy [86]. To date, no studies are available in which omics techniques are applied to seeds to explore the pre-germinative metabolism and the germination performance in response to environmental changes. Such a severe gap of knowledge must be rapidly filled in order to generate novel sources of molecular hallmarks of seed quality.

## 5. Commercial Use of Seed Priming

In recent years, the application of seed priming technology in commercial seed production has developed rapidly at a global level and the number of seed companies using various priming technologies is expanding. The target species include agricultural crops, vegetables, ornamental plants and cover crops for soil improvement. Some companies have also started to produce and process the materials used for seed priming. In this scenario, the commercial sector dedicated to onion and onion-like crops is still limited. As shown in Figure 2, 24 companies working on onion are located in Europe, and Italy stands as the major component (8 companies). Outside Europe, 12 companies are found in the USA, 8 in China, and 6 in India. Only 12 companies working on leek are listed in dedicated websites (e.g., AgriEXPO, https://www.agriexpo.online/agricultural-manufacturer/leek-seed-6435.html, accessed on 19 December 2022). What about the use of seed priming techniques by these companies? Are there commercial, patented products for onion seed quality enhancement that can be used on a large scale? As shown in Table 4, only few formulations are currently available, for both onion and leek, developed by leading Dutch seed companies.


**Figure 2.** Global distribution of breeder/producers of onion seeds. For each country, the number of companies involved in onion breeding and seed production is shown in brackets. Data retrieved from SeedQuest®. (https://www.seedquest.com/seed/vegetables/onion/index.htm, accessed on 19 December 2022).

**Table 4.** Commercial products for onion seed enhancement currently available.

| Product Commercial Name | Target Crop | Description | Company (City, Country) |
|---|---|---|---|
| Promotor onion | onion | tailored priming protocol to improve the speed of emergence and results in more uniform germination | Incotec (Enkhuizen, The Netherlands) |
| Incotec 118 Special | onion | a melting pellet allowing the seed quick access to oxygen, combined with a priming treatment for faster and more uniform germination | Incotec (Enkhuizen, The Netherlands) |
| Promoter leek | leek | priming formulation used to obtain faster, more uniform germination, and stronger crop stand; it broadens the temperature range at which seed will germinate; promotes photo-dormancy and thermo-dormancy release | Incotec (Enkhuizen, The Netherlands) |
| *Promoter Plus Leek* | leek | combination of priming and upgrading technology developed to enhance stand establishment through uniform germination, and to increase speed of emergence; increases the number of usable transplants | Incotec (Enkhuizen, The Netherlands) |
| B-Mox® | onion | seed enhancing formulation, promotes germination and seedling growth (5% improvement compared to stsandard priming treatments) | Bejo (Warmenhuizen, The Netherlands) |

*Challenges, Open Questions, and Perspectives*

Seed priming has different effects on different plant species or even cultivars. The successful output of treatments is strongly genotype- and seed lot-dependent, and in some cases negative effects are observed after priming. Decreased seed storage capacity or/and shorter seed lifespan represent the main drawbacks of the technology. At present, mechanisms underlying the seed response to priming, contributing to define the basics of the technology, are still unclear. Although new seed priming technologies have emerged in recent years, some of them have not yet been validated for commercial use. The most widely used priming methods are based on soaking seeds in solutions of mineral salts. The basic questions to be addressed concern the most suitable use of seed priming, the final costs of large-scale application and the strategies for improving its final performance. All these are still open questions. Therefore, more in-depth basic and applied research is needed to better elucidate the seed priming mechanism and the detection of priming beneficial effects in the field, and then boost its commercial application. For large-scale commercial use, optimal priming methods and conditions tailored for different cultivars or species will require further extensive experimental activity. It is undeniable that seed priming technology has the characteristics of an easy operation, with a low cost and high application value in the industrial and market context. It is foreseeable that this technology will have broader prospects in agricultural production and ecological construction. Furthermore, seed priming can effectively restore or improve seed vigour and ensure the success of germplasm resource conservation and subsequent propagation, particularly for those seeds undergoing severe loss of vigour.

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