

Effect of pre-sowing treatment on seed germination and seedling vigour in endive and chicory

N. G. TZORTZAKIS

Department of Vegetable Science, School of Agricultural Technology, Technological Education Institute of Crete, Heraklion, Greece

ABSTRACT: The influence of pre-sowing treatments using hydropriming, osmopriming and halopriming in laboratory and/or nursery tests on seedling emergence, seedling weight and plant growth of endive and chicory was examined. Halopriming (KNO_3) or growth regulators (gibberelic acid; GA_3) improved the rate of germination of endive and chicory and reduced the mean germination time needed. 30 min pre-sowing treatment with NaHClO_3 , methyl jasmonate and dictamus essential oil decreased seed germination as well as seed radicle length *in vitro*. In the nursery tests, pre-sowing treatments had in some extent impact on the upper part (fresh weight) of the seedling, while no major changes were observed for leaf number and root fresh weight. 6-benzylaminopurine (BAP) or NaHClO_3 treatment reduced plant growth of both species. The present study suggests that KNO_3 and secondly GA_3 treatments may improve rapid and uniform seedling emergence and plant development in nurseries and/or in greenhouses, which is easily applicable by nursery workers with economic profits.

Keywords: emergence; growth regulator; plant growth; seed germination; seed priming

Over the past two decades seed enhancement through seed priming has led to great improvements in a grower's ability to routinely achieve this goal in both the field and greenhouse. Numerous vegetable and ornamental crop species have been primed successfully. In order to maintain a superior product, seed companies have to maintain seed quality and longevity in the primed seed. Rapid and uniform field emergence are two essential prerequisites to increase yield, quality, and ultimately profits in crops. Uniformity and percentage of seedling emergence of direct-seeded crops have a major impact on final yield and quality. Slow emergence results in smaller plants and seedlings, which are more vulnerable to soil-borne diseases. Extended emergence periods predispose the planting bed to deterioration and increased soil compaction, particularly under adverse environmental conditions.

Poor germination is a common phenomenon at sub-optimal temperatures, which is a great concern of growers that grow seedlings in late winter and early spring in the Mediterranean region. Optimum seed germination and seedling emergence occur at relatively high temperatures (20–30°C) for several species (e.g. tomato, eggplant, bean, watermelon, cucumber, melon).

Nowadays, various seed priming techniques have been developed, including hydropriming (soaking in water), halopriming (soaking in inorganic salt solutions), osmopriming (soaking in solutions of different organic osmotica), thermopriming (treatment of seed with low or high temperatures), solid matrix priming (treatment of seed with solid matrices), and biopriming (hydration using biological compounds) (ASHRAF, FOOLAD 2005). Each treatment has advantages and disadvantages and may have varying effects depending upon plant species, stage of plant development, concentration/dose of priming agent, and incubation period. The use of a salt as an osmoticum can lead to an increase in fresh weight of a seed. In this case, germination is delayed through increased solute potential of the embryo. Osmo-conditioning or osmotic conditioning is also used to describe the same treatment when materials such as polyethylene glycol (PEG) are used as the osmoticum. Solid matrix priming (SMP) is a term used for a pre-sowing seed treatment in which a solid-matrix instead of an osmotic solution is used to enhance germination. Matricconditioning was proposed as an alternative term to SMP, to distinguish seed conditioning by matric and osmotic forces. Biopriming is a treatment where sweet corn seeds are coated with a bacterium and soaked in

warm water until the seed moisture content increases to 35–40%.

Seed priming treatments (pre-sowing seed treatment) using moist-chilling, growth regulators (e.g. gibberelic acid applications; GA₃) and plant-derived smoke compounds (EL-DENGAWY 2005; VAN STADEN et al. 2006), magnetic fields (AHMET 2003) and salts such as KNO₃ have been effective in improving seed germination at low and high temperatures (SACHS 1977; NERSON, GOVERS 1986; DEMIR, VAN DE VENTER 1999; DEMIR, MAVI 2004). Seed maturation stage can also be an influential factor in germination performance at low temperatures and response to priming treatment (OLOUCH, WELBAUM 1996; DEMIR, OZTOKAT 2003). In general, mature seeds tend to show a better germination performance at stress temperatures than those of earlier and later harvests, while advancement obtained by priming was greater in earlier harvests (e.g. premature seeds) (OLOUCH, WELBAUM 1996).

Several studies on seed germination and seed emergence revealed the beneficial effects of seed priming by several ways (heat, smoke, soaking, leaching, temperature, scarification and NaCl salinity) (AHMED et al. 2006). Solid matrix priming improved germination of hot pepper seed by 10–16% depending on temperature, and this effect enhanced when SMP was followed by halopriming and osmopriming (PANDITA et al. 2007). Moreover, hydropriming (48 h) for tomato and sand matricpriming (80% water holding capacity, 3 days) for eggplant and chilli were established as best methods of priming treatment capable of improving seed vigour (VENKATASUBRAMANIAN, UMARANI 2007). Previous studies revealed the beneficial effects on seed germination and seedling emergence of priming (– 1.5 MPa KH₂PO₄ solution for 20 h at 15°C, in darkness) lettuce seeds following high temperature (35°C) and/or incorporating plant growth regulators (putrescine or 1-aminocyclopropane-1-carboxylic acid (ACC)) (KORKMAZ 2006). Furthermore, a low frequency magnetic field (16 Hz) can be used as a method of postharvest seed improvement for different plant species, especially for seeds of temperature-sensitive species germinating at low temperatures (ROCHALSKA, ORZESZKO-RYWKA 2005). The effect of magnetic field and/or KNO₃ on asparagus seeds resulted in increased seed germination and seedling growth (epicotyl and hypocotyl length) as well as it changed the direction of radicle growth (SOLTANI et al. 2006).

Increasing of NaCl concentration (up to 200mM) reduced the germination percentage, the growth parameters and the relative water content of four lentil genotypes (SIDARI et al. 2007) and affected seed

germination for several species (carrot, chile pepper, tomato and guayule) (MIYAMOTO et al. 2004). Moreover, several factors (such as dehydration temperature and/or relative humidity) may affect pepper seed emergence and/or germination (DEMIR et al. 2005). High temperatures during sowing may delay or inhibit carrot seed germination. Consequently, seed priming may be used as an important tool to improve seed performance and stand establishment in the field (e.g. carrot), especially during the summer (NASCIMENTO, PEREIRA 2007).

The objectives of the present study are: (i) to develop effective pre-sowing treatments to stimulate seed germination and seedling vigour, and (ii) to identify morphological traits for the assessment of healthy seedlings of endive (*Cichorium endivia* L.) and chicory (*Cichorium intybus* L.). Means of hydropriming, osmopriming and halopriming were employed in laboratory and/or nursery tests.

MATERIAL AND METHODS

Seed source

Seeds of endive (Pancalieri) and chicory (chicory white stem) were purchased from Agrosemi company (Agrosemi Ltd., Athens, Greece) and Agrimore (Agrimore SA, Thessaloniki, Greece), respectively.

Germination and emergence studies in laboratory

For germination tests, air-dried endive and chicory seeds were washed thoroughly with double distilled water (DW), and dipped in various pre-treatment solutions (24 h, 23 ± 2°C, dark). These included gibberelic acid (GA₃; 25 and 250µM); 6-benzylaminopurine (BAP; 25 and 250µM), KNO₃ (50 and 150mM), methyl jasmonate (MJ; 50 and 500µM), dictamnus (*Origanum dictamnus* L.) essential oil (Dict.; 50 and 500µM (extracted by hydrodistillation)) and NaHClO₃ (5% v/v available chlorine for 15 and 45 min). Control was maintained using DW. Treated seeds were washed 2–3 times with DW and placed in Petri dishes lined with Qualigens (615 Å) filter paper (four replicates/treatment, 25 seeds/replicate) in randomized design under laboratory conditions (average temperature: 26.02 ± 2.10°C max., 23.8 ± 2.20°C min.) and monitored daily. The filter papers were moistened daily using DW. Seeds were considered germinated upon radicle emergence; the first germination was

observed after one day. The final germination was obtained before seven days. The mean radicle length (in mm) was evaluated on the seventh day.

Germination, emergence and plant growth studies in nursery tests

Endive and chicory seeds from the same cultivars were used for nursery tests. Only four best treatments from the laboratory experiment were conducted. Ninety-six seeds/treatment were treated for 24 h with DW, GA₃ (250µM), BAP (25µM), KNO₃ (50mM), or with 5% NaHClO₃ for 15 min as mentioned previously. Seeds were sown (0.5 cm depth; distance between seeds 3.0 cm) in plastic (PVP) seedling trays (3 seeds per well; 4 well per replication; five replications per treatment, 30 cm³ well capacity) on top of the surface of medium consisted of mixture 2:1 (v/v) with peat (Professional peat, Gebr. Brill Substrate GmbH & Co. KG, Georgsdorf, Germany) and perlite (Perloflor, Protectivo EPE, Athens, Greece). Perlite physicochemical properties were reported in previous studies (TZORTZAKIS, ECONOMAKIS 2005). The experiment was carried out in completely randomized design in an unheated glasshouse with a north-south orientation at the School of Agricultural Technology in Heraklion, Crete, Greece, located at the latitude of 35°35'N, longitude 24°02'E and altitude 8 m. Temperature was 25.7 ± 6.88°C max., 15.1 ± 5.05°C min.; RH: 81.5 ± 1.93 max., 74.8 ± 4.09 min., with alternate-day watering. Daily observations recorded the first germination (seeds recorded as emerged when the hypocotyls appeared above the surface of substrate medium) after four days of sowing; the first true-leaf emerged after eight-days. After 15 days, seedlings were thinned to single plant to mitigate competition, maintaining 8–10 cm distance. After 28 days, seedling growth was assessed by harvesting five individuals per treatment. Seedlings were counted, cleaned, and upper-part and root fresh weight (g/plant) as well as the root length (cm/plant) and the greater leaf length (cm) were determined. Mean germination time (MGT) was calculated as follows, according to LABOURIAU (1983):

$$t = \frac{\sum ni \times ti}{\sum n} \text{ (days)}$$

where:

t – mean germination time,

ti – given time interval,

ni – number of germinated seeds during a given time interval,

n – total number of germinated seeds.

The experimental design was in duplicate. Percentage data were log-transformed before analysis. The growth parameters of seedlings were subjected to one-way analysis of variance (ANOVA) employing Duncan's multiple range test (Duncan's MRT) at *P* = 0.05 in SPSS (SPSS, v. 11, Chicago, IL). Graphs were produced using Prism v.2.0 (Graph Pad Inc., San Diego, CA).

RESULTS AND DISCUSSION

Seed germination *in vitro*

Among the 13 laboratory treatments, few of them significantly stimulated seed germination (Fig. 1). The presence of KNO₃ (50mM and 150mM) was significantly (*P* < 0.05) effective, possibly through oxidized forms of nitrogen causing a shift in respiratory metabolism to the pentose phosphate pathway (ROBERTS, SMITH 1977). GA₃ increased seed germination (32%) at the first two days, thus confirming its role as a stimulatory agent. 30 min pre-sowing with NaHClO₃ decreased seed germination (up to 34%), contrasting with the stimulatory effects reported by others (HSIAO 1979; HO et al. 1995; NADEEM et al. 2000); this may be related to the type of seed (plant species) and/or concentration/time depended. These stimulatory treatments may help germinating seedlings early, providing them higher competitive ability (ZHANG, MAUN 1990) and hence reducing chances of their mortality.

Seed radicle length (mm) following pre-sowing treatment was reduced for 500µM MJ (up to 97%), 30 min NaHClO₃ (up to 92%), 250µM BAP (up to 73%), 500µM Dict. (up to 58%) and 25µM BAP (up to 42%), whereas no differentiation was observed among the others treatments (Fig. 2). The increased concentration of MJ or dictamus oil, possibly causing toxicity effects, was accompanied with brownish radicles (Fig. 3). Similarly, oils from *Artemisia princeps* Pamp and *Cinnamomum camphora* (L.) Presl applied individually were significantly toxic to seed germination of wheat at 500 µg/ml. However, no toxic effects were found when the two oils were mixed (1:1 w/w) at the same concentration (LIU et al. 2006), and this may be applied in future studies.

Seed germination and emergence *in vivo*

Under nursery conditions, pre-sowing treatment with GA₃ stimulated seed germination of endive and (including KNO₃ treatment for chicory) (see Figs. 4

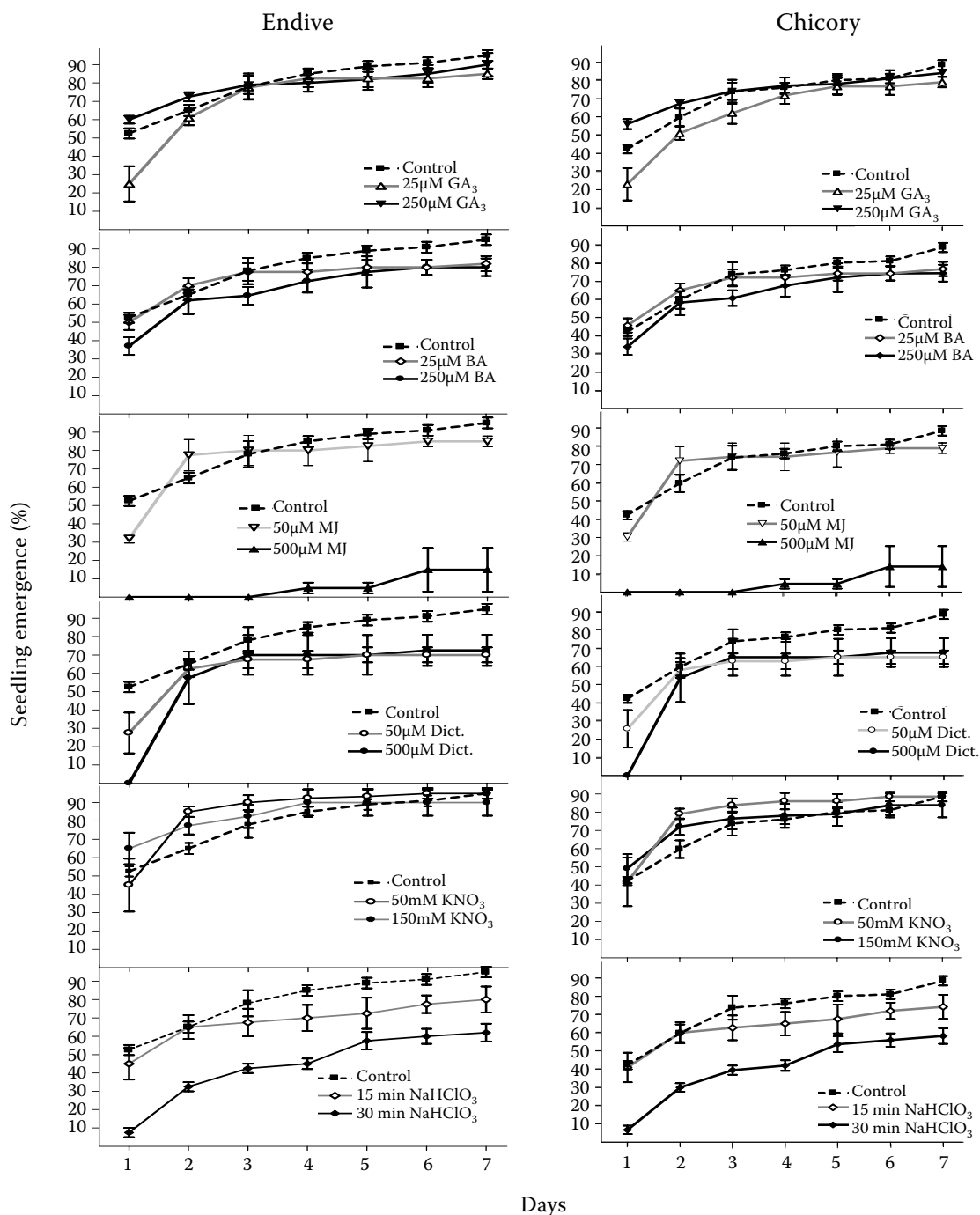


Fig. 1. Cumulative seedling emergence of pre-sowing treated (GA_3 , BAP, MJ, Dict., KNO_3 and NaHClO_3) and control endive and chicory seeds germinated *in vitro*. Values represent mean (\pm SE) of measurements made on four independent Petri dishes per treatment

and 5) thus reduced MGT over control. NaHClO_3 treatment was ineffective or reduced (up to 66%) seed germination whereas BAP suppressed seed germination between 34% (for chicory) and 70% (for endive) which resulted in the greatest MGT values (from 12 to 14 days). Untreated seeds started germination/emergence by the 4th day. Thus, the stimulation of treated chicory seed comparing with untreated seeds might be due to altered physiol-

ogy of embryos and activation of enzymes, so that developmental processes occur more rapidly after sowing (KATTIMANI et al. 1999).

Seedling growth

Analyses of variance showed that pre-sowing treatment with KNO_3 significantly enhanced plant

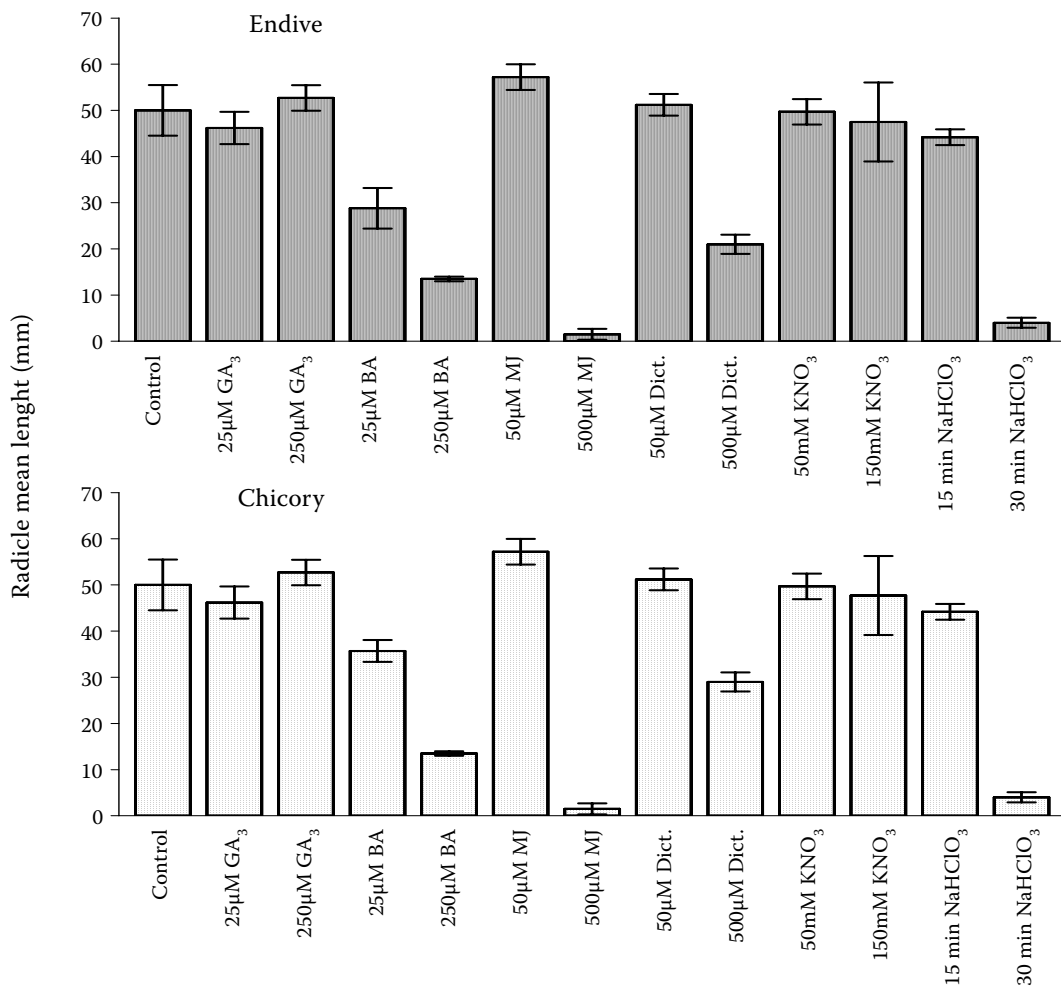


Fig. 2. Endive and chicory seed radicle length (mm) following pre-sowing treatment (GA₃, BAP, MJ, Dict., KNO₃ and NaHClO₃) *in vitro*. Values represent mean (± SE) of measurements made (four radicle/dish) on four independent Petri dishes per treatment

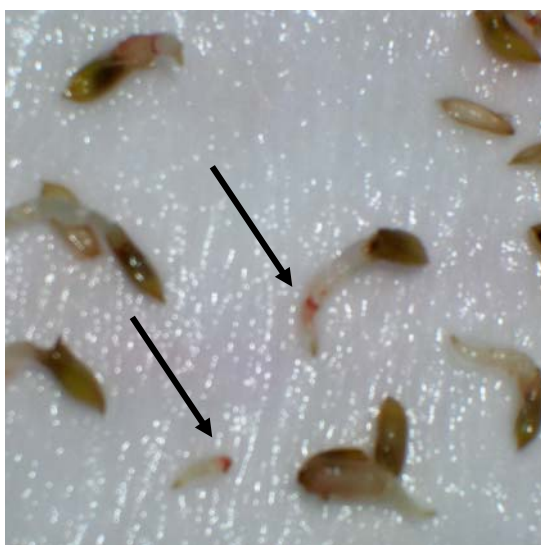


Fig. 3. Brownish of seed radicles following pre-sowing treatment for 24 h with 500µM MJ

growth (shoot and root fresh weight) of both species, whereas no differences were observed for leaf number and leaf size (Tables 1 and 2). Moreover, no differences were observed for root length following pre-treatment with KNO₃ or GA₃. However, root elongation in other reported plants (medicinal) was positively influenced (KATTIMANI et al. 1999) by KNO₃ and negatively (NAUTIYAL et al. 1985) by GA₃ application. BAP or NaHClO₃ treatment reduced plant growth of both species. In previous studies, germination of *Amaranthus cruentus* L. seeds at 20°C was promoted by priming in 3% KNO₃ but not in KH₂PO₄ or PEG solutions. Inclusion of MJ, ACC, BAP or spermine into priming solution further improved germination (TIRYAKI 2006). Similarly, employment of gibberellic acid and/or glycinebetaine enhanced rice seed emergence and seedling growth (CHEN et al. 2005). Furthermore, when float-system was employed, it was revealed that nutrient solution application

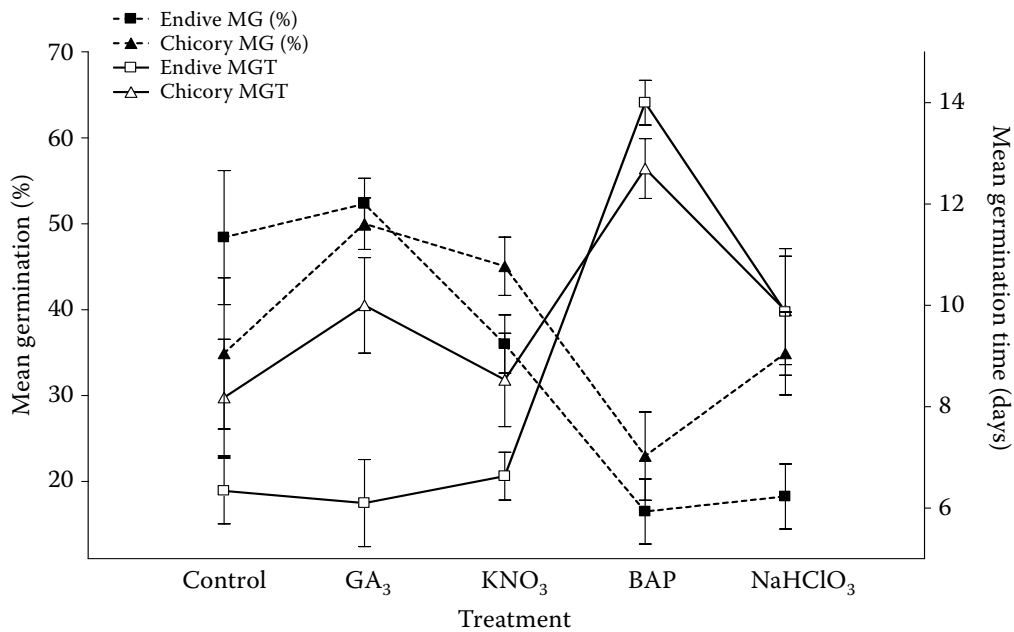


Fig. 4. Mean seed germination (MG) and mean germination time (MGT) in endive and chicory using various pre-sowing chemical treatments under nursery condition. Values represent mean (\pm SE) of measurements made on twenty independent wells of seedling tray per treatment

and/or constant heating of liquid media at 25°C induced seedling biomass and leaf length but no major changes were observed in root length for a variety of plant species examined, including leafy vegetables (TZORTZAKIS, ECONOMAKIS, unpublished data).

For endive, leaf length showed negative relationship with upper fresh weight for KNO₃ pre-sowing treatment ($y = -1.281x - 0.78$) under highly significant correlation ($r^2 = 0.95$) but revealed positive re-

lationship ($y = 0.071x - 0.01$; $r^2 = 0.82$) for NaHClO₃. No correlation was observed between leaf length and root fresh weight. In case of chicory, the only linear relationship was revealed between leaf length and root fresh weight for BAP pre-sowing treatment ($y = 0.201x + 4.98$) that were also closely correlated ($r^2 = 0.87$).

Seed emergence losses in the soil are not generally caused by germination failure, but by the failure of seedlings to grow and emerge above soil surface

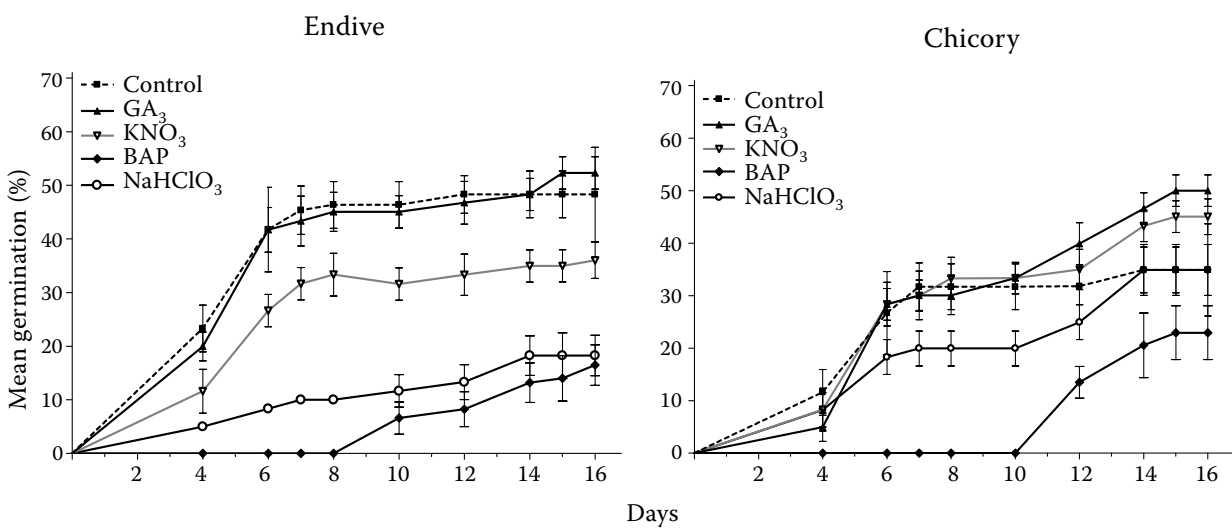


Fig. 5. Cumulative seedling emergence of pre-sowing treated (GA₃, BAP, KNO₃ and NaHClO₃) and control endive and chicory seeds germinated *in vivo*. Values represent mean (\pm SE) of measurements made on twenty independent wells of seedling tray per treatment

Table 1. Effect of pre-sowing chemical (GA_3 , KNO_3 , BAP or $NaHClO_3$) treatments on seedling growth (seedling upper part and root fresh weight (g/plant) as well as the root length (cm/plant), number of leaf produced and leaf length (cm/plant)) of endive under nursery condition (greenhouse) after 16 days of sowing

	Root fw* (g)	Upper fw* (g)	Root length (cm)	Leaf No.	Leaf length (cm)
Control	6.31 ^a	5.25 ^b	39.3 ^a	6.8 ^a	19.6 ^a
GA_3	5.07 ^a	5.39 ^b	35.5 ^a	6.8 ^a	19.3 ^a
KNO_3	6.88 ^a	8.30 ^a	43.1 ^a	7.8 ^a	19.9 ^a
BAP	2.07 ^b	4.04 ^b	23.6 ^b	6.8 ^a	17.9 ^a
$NaHClO_3$	0.37 ^b	0.47 ^c	17.9 ^b	3.0 ^b	6.9 ^b

* fresh weight; in each column, values followed by the same letter did not differ significantly ($P < 0.05$) according to Duncan's multiple range test

Table 2. Effect of pre-sowing chemical (GA_3 , KNO_3 , BAP or $NaHClO_3$) treatments on seedling growth (seedling upper part and root fresh weight (g/plant) as well as the root length (cm/plant), number of leaf produced and leaf length (cm/plant)) of chicory under nursery condition (greenhouse) after 16 days of sowing

	Root fw* (g)	Upper fw* (g)	Root length (cm)	Leaf No.	Leaf length (cm)
Control	3.66 ^b	4.70 ^{bc}	32.3 ^a	5.4 ^{bc}	21.6 ^{ab}
GA_3	3.94 ^{ab}	7.91 ^a	29.3 ^a	7.0 ^a	22.2 ^a
KNO_3	5.38 ^a	6.55 ^a	35.1 ^a	6.2 ^{ab}	22.9 ^a
BAP	0.88 ^c	2.67 ^c	13.2 ^c	4.0 ^c	18.4 ^c
$NaHClO_3$	3.81 ^b	3.40 ^{bc}	17.9 ^b	5.0 ^{bc}	19.3 ^{bc}

* fresh weight; in each column, values followed by the same letter did not differ significantly ($P < 0.05$) according to Duncan's multiple range test

(HALMER, BEWLEY 1984). Soil salinity may affect the germination of seeds either by creating an osmotic potential external to the seed preventing water uptake, or through the toxic effects of Na^+ and Cl^- ions on the germinating seed (PEREZ et al. 1998; KHAJEH-HOSSEINI et al. 2003). Salt and osmotic stresses are responsible for both inhibition or delayed seed germination and seedling establishment (ALMANSOURI et al. 2001). Under these stresses there is a decrease in water uptake during inhibitions and furthermore, salt stress may cause excessive uptake of ions (MURILLO-AMADOR et al. 2002).

Nowadays, various seed priming techniques have been developed, including hydropriming, halopriming, osmopriming, thermopriming, solid matrix priming, and biopriming (ASHRAF, FOOLAD 2005). Each treatment has its advantages and disadvantages and may have varying effects depending upon plant species, stage of plant development, concentration/dose of priming agent, and incubation period. Indeed, growers have great concern for uniform and rapid seedling emergence and plant development in late

winter and early spring in the Mediterranean region in order to reach local and North Europe demands.

The present study suggests that KNO_3 and secondly GA_3 treatments may improve rapid and uniform seedling germination-emergence and plant development in nurseries and in greenhouses. They are economic and easily applicable by nursery workers and poor farmers in developing mass planting stock, compared to costly plant growth regulators and associated technicalities. In cases with great percentages of seed germination failure, further studies are required for alternative treatments (optimizations of temperatures, substrates etc., and/or combining of seed priming techniques).

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Vliv ošetření semen před setím na jejich klíčivost a vitalitu semenáčků u endivie a čekanky

ABSTRAKT: U čekanky a endivie byl testován vliv ošetření semen před setím vodou a roztoky organických osmotik i anorganických látek na vzházení, hmotnost semenáčků a další růst rostlin, a to jak v laboratorních testech, tak při výsevu do školek. Působení roztoku KNO_3 a růstového regulátoru (kyselina giberelová, GA_3) zlepšilo klíčivost semen endivie a čekanky a snížilo průměrnou dobu potřebnou ke klíčení. Třicetiminutové ošetření NaHClO_3 , metyljasmonátem a diktamovým esenciálním olejem snížilo klíčivost semen i délku radikuly *in vitro*. Ve školkových testech úpravy semen před setím do jisté míry ovlivnily i čerstvou hmotu nadzemní části semenáčků, zatímco nebyly zaznamenány žádné významné změny v počtu listů a čerstvé hmotě kořenů. Při použití 6-benzylamino-purinu (BAP) nebo NaHClO_3 došlo k redukci růstu rostlin u obou druhů. Práce naznačuje, že použití KNO_3 nebo případně GA_3 může urychlit klíčení osiva a zlepšit synchronizaci klíčení i vývoj rostlin ve školkách i ve skleníku, což přinese ekonomický profit.

Klíčová slova: vzházení; růstový regulátor; růst rostlin; klíčení osiva; ošetření osiva

Corresponding author:

Dr. NIKOS G. TZORTZAKIS, Technological Education Institute of Crete, School of Agricultural Technology, Department of Vegetable Science, 71004 Heraklion, Greece
fax: + 30 2810 318 204; e-mail: ntzortzakis@Gmail.com
