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Vermicompost Leachate as a Promising Agent for Priming and Rejuvenation of Salt-Treated Germinating Seeds in *Brassica Napus*

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ABSTRACT

Salinity is a major constraint hampering germination and early seedling growth, especially in aged seed lots. Any rejuvenation treatment improving salt resistance at these crucial developmental stages will be of special interest. Two sets of experiments were performed in *Brassica napus* to precise the impact of vermicompost leachate (VCL) on seed germination in the presence of NaCl and to analyze its putative interest as seed priming agent before NaCl exposure. Two seed lots were used: one old seed lot (cv. Libomir) and a recent one (cv. Harry). VCL increased the germination percentage of aged seeds in the absence of NaCl and increased seedling length in both cultivars. VCL had only a minor impact when directly added to the NaCl-containing germinating solution. In contrast, priming with VCL strongly improved subsequent germination in the presence of NaCl in relation to a more efficient management of oxidative stress in both cultivars. The improvement of salinity resistance provided by VCL priming was not due to modification in ion or proline content. It is concluded that VCL may act as a rejuvenation agent invigoring old seed lots and as an efficient priming agent for improvement of salinity resistance at the germination stage. Valuable properties of VCL are discussed in relation to the simultaneous presence of several protecting compounds.

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Germination; phytohormones; salinity; vermicompost

Introduction

Salt stress is a major environmental constraint limiting plant growth and yield in numerous cultivated areas in the world. It induces a wide range of physiological disorders in relation to a decrease in water availability due to the low osmotic potential of the soil solution, accumulation of toxic ions, oxidative stress, decrease in net photosynthesis, alteration in the plant hormonal status and morphological perturbations (Ashraf and Harris 2004; Haddadi, Hassanpour, and Niknam 2016; Munns 2002; Munns and Tester 2008; Parida and Das 2005; Yildiz and Terzi 2013).

Plant responses to salinity vary according to the developmental stage. Although germination by itself is not the most salt-sensitive stage of the plant's cycle in glycophyte species, moderate salinities were reported to delay germination while higher doses of NaCl irreversibly reduce the final germination percentage (Almansouri, Kinet, and Lutts 2001; El-Shaieny 2015; Lutts, Kinet, and Bouharmont 1995). Salinity affects germination by limiting water absorption by the seeds, reducing mobilization of stored reserves or by directly affecting the structural organization, protein synthesis, DNA replication, cell division and cell elongation in germinating embryos (Almansouri, Kinet, and Lutts 2001; Daszkowska-Golec 2011; Marconi et al. 2013). Young seedlings issued from germination are extremely sensitive to salt stress, especially if germination occurs in surface soils which accumulate soluble salts as a consequence of

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evaporation and capillary rise of water (Bajji, Kinet, and Lutts 2002; Bewley, Bradford, and Hilhorst 2012; El-Shaieny 2015; Mahmoudi et al. 2011; Sarker, Hossain, and Kashem 2014).

Seed priming is a pre-sowing treatment allowing the seeds to encounter a limiting hydration process that does not allow radicle protrusion during the reversible phase of germination (Lutts et al. 2016). During priming, seeds may be exposed to low doses of salts (Afzal et al. 2008), osmotic agent such as polyethylene glycol (PEG) (Chen and Arora 2011; Pradhan et al. 2014), biotic agent such as plant growth promoting bacteria (Kaymak et al. 2009), phytohormones or related compounds (Afzal et al. 2009; Jisha and Puthur 2016; Younesi and Moradi 2014; Zhang et al. 2007). The primed seeds may be dehydrated and stored until final sowing. They usually exhibit a higher level of stress resistance at both the germination and young seedling stage as well as a more synchronous germination (Afzal et al. 2008; Ashraf, Arfan, and Ahmad 2003; Basra et al. 2003; Pradhan et al. 2014). In most if not all cases, one single agent is added to the priming solution and classical approaches thus do not consider the complementary advantages of different compounds acting in a positive synergistic way (Lutts et al. 2016). Vermicompost is a biologically active organic material resulting from the degradation of organic residues by earthworms and associated microorganisms (Ibrahim, Quaik, and Ismail 2016). Its liquid fraction, known as "vermicompost tea" or "vermicompost leachate" (VCL) is a valuable source of nutrients, phytohormones, humic acids and antioxidants (Aguiar et al. 2013; Aremu et al. 2015; Zhang et al. 2014). Vermicompost and its derivatives are widely used to improve plant production (Chamani, Joyce, and Reihanytabar 2008; Singh et al. 2008; Tejada et al. 2008). Ievinsh (2011) and Arancon et al. (2008) demonstrated that vermicompost improved seed germination and early seedling growth. According to Amorim et al. (2015), germination improvement is mainly attributable to the positive impact of humic acids. To the best of our knowledge, impact of VCL on germination in the presence of NaCl remains poorly documented. Moreover, VCL was never tested as a seed priming agent for the subsequent improvement of seeds germinating in salt stress conditions.

Rape (*Brassica napus*) is one of the most important crops in the world and is now the third largest source of edible oil cultivated on more than 26 million hectares. It belongs to the same family as the plant model species *Arabidopsis thaliana* with a completely sequenced and annotated genome. In this species, salt and drought stress are the main limiting factors reducing seed germination and capacity and contribute to a strong decline in yield (Tan et al. 2017). Priming treatments were demonstrated to have a positive impact on rapeseed germination (Benincasa et al. 2013; Kubala et al. 2015a, 2015b; Lechowska et al. 2019) but, to the best of our knowledge, VCL priming was never tested in this important species. Moreover, studies dealing with priming processes for salt-tolerance improvement commonly use seed lots with the highest germination percentage in control conditions. Beside external constraints, seed aging represents an internal factor hampering seed germination, and is especially important to consider in countries where commercial seeds are expensive and storing conditions are not optimal. Seed aging and viability are indeed affected by damages occurring during storage (Bewley, Bradford, and Hilhorst 2012). Any strategy increasing the germination percentage of old seed lots is important to consider. Priming efficiency may also vary depending on the age of the seeds but interaction between aging and priming for salinity tolerance is poorly documented.

The aim of the present work was to i) analyze the impact of vermicompost leachate added directly to germinating medium containing NaCl ii) to precise its putative interest as a priming agent to improve subsequent germination in salt stress conditions and iii) to compare the effects of VCL on two seed lots of canola differing for their mean level of viability.

Materials and methods

Plant material and vermicompost leachate

Seeds of *Brassica napus* L. (cv. Libomir and cv. Harry) used in this study were kindly provided by Dr. L. Wojtyla (Adam Mickiewicz University of Poznan, Poland). Seeds from cv. Libomir were produced in 2007 and those of cv. Harry were produced in 2016. Accordingly, when germination was performed

Table 1. Chemical properties, nutrients concentration, and phytohormones contents in vermicompost leachate resulting of food waste vermicomposting (n = 3). ND: not detected. Soluble sugars, phenolics, and humic acids were determined according to Benhebil (2013) while polyamines and phytohormones were quantified according to Gharbi et al. (2017).

Constituent	VCL
pH	8.78
Density	1.006
EC (mS/cm)	20.4
Osmpotic potential (Ψs; MPa)	−0.59
Soluble sugars (µg/ml)	290
Polyphenols (µg/ml)	27.11
Anthocyanes (µg/ml)	8.47
Humic acid (mg/l)	678
Na^{+} (mg/ml)	0.907
K^{+}	3.543
Mg^{+2}	0.158
Ca^{+2}	0.157
Putrescine (nmol/ml)	8.83
Spermine	12.39
Spermidine	12.91
Total cytokinins (pmol/ml)	11.91
Total auxins	17.20
Abscisic acid and ABA metabolites	13.74
Jasmonates	1.46
Salicylic acid	164.89
Phenylacetic acid	5.24
Benzoic acid	449.73
1-aminocyclopropane-1-carboxylic acid	196.99
Gibberellins	ND

under standard conditions according to the International Seed Testing Association Rules (ISTA 2018): the germination percentage for cv. Harry varied between 93.4% and 97.2% while the germination percentage of cv. Libomir never exceeded 30% (Lutts, unpublished results).

Vermicompost leachate was produced by the biotechnology laboratory in the University of Blida (Algeria) through the activity of earthworms from food waste as detailed by Benhebil (2013). The main chemical properties, nutrients and phytohormone concentration quantified in the vermicompost are presented in Table 1.

Germination tests

Two sets of experiments were performed. The first one (Experiment 1) analyses the direct impact of VCL added to germinating solution in the presence and absence of NaCl. The second set of experiments (Experiment 2) aimed to determine the impact of VCL used as a priming agent on subsequent germination of seeds exposed or not to NaCl during germination.

For Experiment 1, seeds of each cultivar were surface-sterilized and placed on Petri Dishes lined with three layers of filter paper Whatman no. 2 imbibed with 6 ml of sterile deionized water (control), NaCl (100 mM), vermicompost leachate (1:10, v/v) (VCL) and a combination of NaCl/VCL .

For Experiment 2, the seeds of each cultivar were soaked in vermicompost leachate solution (1:10, v/v) for 48 h (VCL-priming) using a volume of 1 ml of priming solution for 2 g of seeds. Such a ratio allows seed imbibition but prevents radicle protrusion. Priming treatment was conducted in darkness under 23°C. Seeds were then washed three times with sterile deionized water and re-dried back under a sterile laminar flux until they reach their initial moisture level. For each cultivar, four treatments were defined (1):unprimed seed germinated in water (UP_{H2O}), (2): unprimed seed germinated in NaCl (100mM) (UP_{NaCl}), (3) vermicompost-primed seed germinated in water (VCP_{H2O}) and (4): vermicompost-primed seed germinated in NaCl (VCP_{NaCl}).

For both experiments and for each treatment, 50 seeds in six replicates were incubated at darkness at 23°C. Seed was considered germinated when the radical procured the seed coat. The germinated seeds were counted every day until four days. Different germination parameters were determined:

Germination index (GI) was calculated as described by the Association of Official Seed Analysis (A.O.S.A 1983) using the following formula:

$$GI = \Sigma(Ni/Ti)$$

where Ti is the number of day after sowing and Ni is the number of germinated seeds on the i^{th} day.

The total germination (TG) percentage measured at the fifth day according to:

$$TG(\%) = (totalnumberofgerminatedseeds/totalnumberofseeds) * 100$$

Germination speed was estimated by the time required to reach 50% of germination (T50) and was calculated according to modified formula of Farooq et al. (2005):

$$T50 = t_i + \left((N/2 - n_i)/\left(n_j - n_i\right) * \left(t_i - t_j\right)\right)$$

where N is the final number of germinating seeds and n_j and n_i are the cumulative number of seeds germinated by adjacent counts at times t_j and t_i, respectively, when $n_i < N/2 < n_j$.

Morphological and biochemical analysis were performed on 7-days-old seedlings issued from germination. For dry weight determination, young seedlings (hypocotyls + radicle) were oven-dried at 70°C for two days after fresh weight estimation.

Ion analysis

The dried plant material (seedlings; 50–100 mg DW) were ground and digested in 4 ml of 35% HNO_3 at 80°C until complete evaporation. The residues were dissolved with aqua regia (HCl 37%: HNO_3 67% 3:1) and filtered on Whathman n°1 filter paper. Na^+ and K^+ were quantified by flame atomic absorption spectrophotometer (Thermo Scientific ICE 3300; Waltham, MA)

Proline and malondialdehyde

Proline was quantified according to Bates, Waldren, and Teare (1973). Seeds and young seedlings were quickly frozen in liquid nitrogen and then stored at −80°C until final analysis. Fresh material (*c. a*. 0.25 g) was homogenized in 10 ml (3%) sulfosalicylic acid and centrifuged at 10,000 *g* for 10 min. The supernatant (0.5 ml) was mixed with 1 ml of glacial acetic acid and 1 ml of 2.5% acid ninhydrin (2.5 g of ninhydrin dissolved in a mixture of 60 ml glacial acetic acid and 40 ml 6 M phosphoric acid). The samples were incubated for 1 h at 100°C, and the reaction was stopped by cooling in an ice bath. The reaction mixture was extracted with 2 ml of toluene, mixed vigorously with test tube stirrer, and the chromophore-containing toluene was warmed at room temperature. Absorbance was read at 520 nm using toluene as a blank with a Shimadzu UV-1800 spectrophotometer and converted using a standard curve performed with L-proline (SigmaChemical).

Malondialdehyde (MDA) is commonly considered as an indicator of oxidative stress and was quantified in the seeds and seedlings using the method of Heath and Packer (1968). Frozen samples (0.25 g) were homogenized in 5 ml of trichloroacetic acid (TCA 5% w: v) and centrifuged at 12,000 *g* for 15 min at 4°C. Two ml of thiobarbituric acid (TBA 0.67% w: v) were added to 2 ml aliquot of supernatant and samples were heated to 100°C for 30 min and then rapidly cooled. The absorbance was read at 532 nm. The results were corrected by subtracting the non-specific absorbance component as measured at 600 nm. The concentration of MDA (nmol g^{-1} DW) was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1}.

Statistical treatment

Each experiment was repeated three times and provided similar trend. Percentage data were transformed into arcsine square roots before statistical analysis to ensure homogeneity of error variance. Shapiro-Wilk and Bartlett/Levene test were performed to check normality and variance equality of the data, respectively. When required, values were log-transformed to normalize the frequency distribution. Data were subjected to one-way analysis of variance (ANOVA). The statistical significance of the results was analyzed by the student-Newman-Keuls test at $P < 0.05$. The data were subjected to ANOVA tests using SYSTAT version 12, and are presented as means ± standard errors.

Results

Experiment 1

Germination percentage

As expected, the seeds of the two considered cultivars exhibited contrasting germination percentages in control conditions (Figure 1): the final germination percentage of Libomir (Figure 1(a)) was 35.7% while seeds of cv. Harry presented a germination percentage higher than 90% (Figure 1(b) and Table 2). Addition of VCL in the absence of NaCl significantly increased germination percentage of cv. Libomir which rose to 50.67% after 96 h (Table 2). In the absence of NaCl, VCL also slightly increased the germination percentage of cv. Harry. Salinity reduced the germination percentage of cv. Libomir both in the presence and absence of NaCl while it only delayed germination in cv. Harry, as indicated by the recorded increase in T50, without affecting the final germination percentage which still reached 91.25% after 96 h. In cv. Libomir, addition of VCL to salt reduced germination percentage after 60 h but significantly increased it after 96 h. A similar decrease in germination was observed for cv. Harry after 48 h in VCL+NaCl treatment comparatively to NaCl treatment (Figure 1(b)), and final germination percentage was slightly reduced comparatively to VCL treatment in the absence of salt (Table 2).

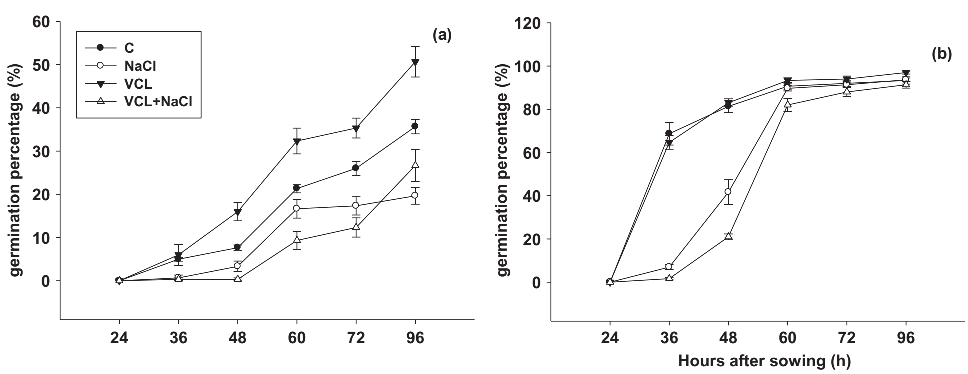

Figure 1. Germination percentage of seeds of *Brassica napus* cvs. Libomir (a) and Harry (b) sown on filter paper imbibed with distilled water (control), 100 mM NaCl solution (NaCl), vermicompost leachate (1:10, v:v) (VCL) and NaCl+VCL solution (1:10,v:v, 100mM) during 4 days. Values are means of six replicates and vertical bars are SE. Each replicate consists in a batch of 100 seeds.

Table 2. Germination parameters (total germination (TG %), germination index (GI) and time to reach 50% germination (T50)) of seeds from cvs. Libomir and Harry sown on filter paper imbibed with water (control), 100mM NaCl solution (NaCl),vermicompost leachate (1:10. v:v) (VCL) and NaCl +VCL solution (1:10. v:v. 100mM).Values are means of six replicates of 100 seeds per replicate ± SE and different letters indicate significant differences between different treatments according to Student-Newman-Keuls test at $P < 0.05$.

	Libomir			Harry		
Cultivar	TG (%)	GI	T50 (d)	TG (%)	GI	T50 (d)
Control	29.58 ± 0.77 b	7.35 ± 0.57 b	5.95 ± 0.18 b	92.92 ± 0.42 b	38.88 ± 0.19 b	1.53 ± 0.00 c
NaCl	19.17 ± 0.53 c	4.47 ± 0.16 c	8.12 ± 0.15 a	91.25 ± 1.07 b	29.63 ± 0.71 c	2.05 ± 0.08 b
VCL	50.67 ± 3.53 a	16.22 ± 1.19 a	4.01 ± 0.21 c	97.00 ± 0.68 a	48.54 ± 0.68 a	1.59 ± 0.01 c
VCL + NaCl	26.67 ± 0.80 bc	5.47 ± 0.80 c	7.42 ± 0.80 ab	91.33 ± 1.52 b	31.33 ± 0.62 c	2.38 ± 0.01 a

Seedling biomass

In cv. Libomir, the treatments had no impact on seedling fresh or dry weight (Table 3). Salinity decreased seedling elongation while vermicompost in the absence of salt strongly increased it. Seedlings exposed concomitantly to NaCl and VCL remained as short as those exposed to NaCl in the absence of VCL. Fresh weight and dry weight of young seedlings of cv. Harry were not affected by the treatment (Table 3) but the length of seedlings was higher in response to VCL application. Salinity reduced elongation in seedling of cv. Harry and this deleterious impact was not mitigated by addition of VCL.

Sodium accumulated in seedlings germinated in the presence of NaCl and to a higher extent in cv. Harry than in Libomir (Figure 2(a)). A slight accumulation of Na^+ was also recorded for Harry germinating in the presence of VCL but in the absence of NaCl comparatively to controls while this effect was not significant for Libomir. The addition of VCL in the presence of NaCl decreased Na^+ accumulation by seedlings of cv. Harry but had no impact in Libomir (Figure 2(a)). Salt stress did not affect K^+ content in cv. Harry (Figure 2(b)) but significantly decreased it in seedlings of cv. Libomir. The presence of VCL induced a conspicuous accumulation of K^+ both in the absence and in the presence of NaCl.

Proline concentration of germinated seedlings remained statistically constant in cv. Harry, whatever the treatment (Figure 2(c)). In cv. Libomir, proline concentration drastically increased in seedlings exposed to salinity and the recorded increase was even higher for seedlings exposed to concomitantly to NaCl and VCL (Figure 2(c)). The MDA concentration also remained unaffected by the treatment in cv. Harry (Figure 2(d)). Salinity increased the MDA concentration of seedlings from cv. Libomir but this effect was more marked in the absence than in the presence of VCL.

Experiment 2

Germination percentage

In Experiment 2, seeds of both cv. were primed with VCL before exposure to salt stress. Unprimed seeds of cv. Libomir germinating in the absence of NaCl still exhibited a germination percentage lower than 40% (Figure 3). It is noteworthy that seeds primed by VCL and subsequently germinating, after drying, in water in the absence of NaCl were able to recover a germination percentage

Table 3. Fresh and dry weight (mg/plant) and length (cm/plant) of seedling grown from germinated seeds of cvs. Libomir and Harry after 7 days on water (control), 100 mM NaCl solution (NaCl), vermicompost leachate (1:10, v:v) (VCL) and NaCl +VCL solution (1:10, v:v. + 100 mM) . Values are means of six replicates ± SE. Different letters indicate significant differences between different treatments according to Student-Newman-Keuls test at $P < 0.05$.

	Libomir			Harry		
Cultivar	FW (mg)	DW (mg)	L (cm)	FW (mg)	DW (mg)	L (cm)
Control	52.35 ± 0.54 a	3.57 ± 0.20 a	7.33 ± 0.60 b	50.79 ± 1.30 a	2.60 ± 0.05 a	10.33 ± 0.60 b
NaCl	58.69 ± 2.24 a	2.85 ± 0.18 a	5.66 ± 0.37 c	39.48 ± 0.51 a	2.23 ± 0.11 a	7.34 ± 0.39 c
VCL	49.09 ± 6.61 a	3.57 ± 0.60 a	10.07 ± 0.45 a	61.90 ± 1.88 a	3.51 ± 0.29 a	11.54 ± 0.42 a
VCL+NaCl	61.67 ± 7.36 a	4.33 ± 0.33 a	5.46 ± 0.31 c	65.71 ± 3.38 a	3.22 ± 0.11 a	8.29 ± 0.39 c

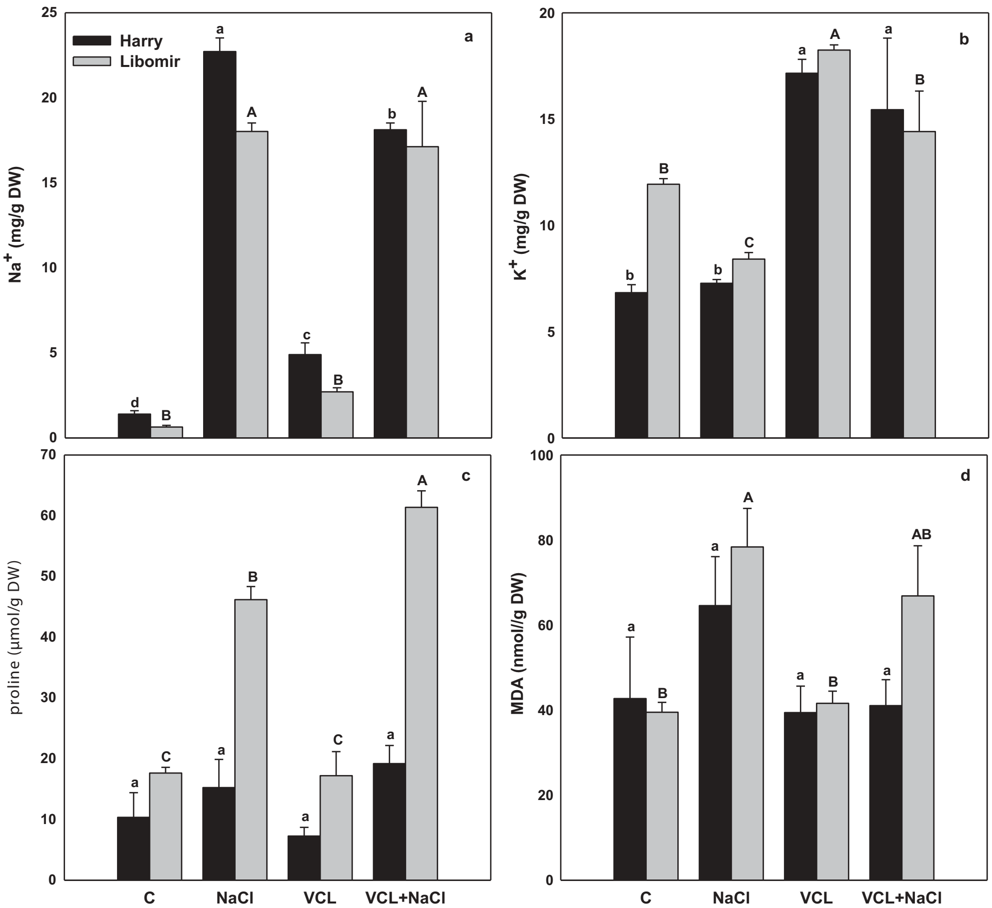

Figure 2. Sodium (a), potassium (b), proline (c) and malondialdehyde (MDA; d) concentration of seedlings from *Brassica napus* issued from seeds of cvs. Harry and Libomir during 7 days on water (c), 100 mM NaCl (NaCl), vermicompost leachate (1:10, v:v) (VCL) and vermicompost + NaCl (1:10,v:v, 100mM) (NaCl+VCL). Values are means of three replicates ± SE and different letters indicate significant differences between different treatments according to Student-Newman-Keuls test at $P < 0.05$. ERach replicate consists in a lot of 20 seedlings.

higher than 90% (Table 4). Primed seeds also germinated more quickly than unprimed seeds as indicated by a significant lower T50 values than unprimed seeds (Table 4). Salinity clearly reduced the final germination percentage and germination indexes in seeds of cv. Libomir and increased the T50 values. Preliminary priming with VCL clearly improved all recorded parameters for this precise cultivar. Priming with VCL also increased the germination speed in seeds of cv. Harry since most seeds germinating in water reach germination within 24 h. A similar effect was recorded for primed seeds of cv. Harry germinating in the presence of salt. In cv. Harry, priming with VCL also slightly increased the final germination percentage (Table 4).

Seedling biomass

In Libomir, salinity had not impact on the weight of seedlings issued from primed and unprimed seeds (Table 5). Priming with VCL decreased FW but increased the length of the obtained seedlings, this effect being similar in the presence and in the absence of NaCl. In cv. Harry, NaCl slightly reduced seedling fresh and dry weight from primed seeds only. The total seedling length was reduced in response to NaCl but was always higher for seedlings obtained from primed seeds.

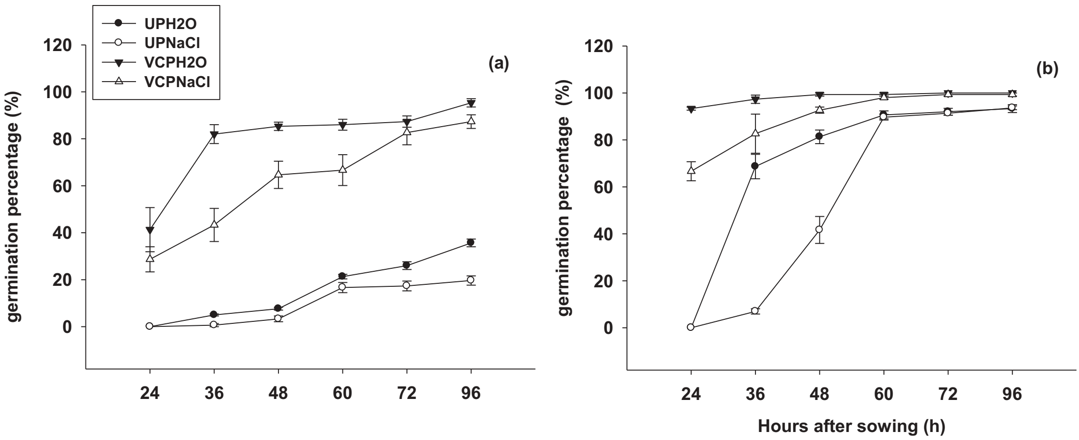

Figure 3. Germination percentage of *Brassica napus* seeds from cvs. Libomir (a) and Harry (b). Seeds were unprimed (UP) or primed in vermicompost leachate (1:10, v:v) (VC-priming VCP), and then sown on filter paper saturated with water (H_2O) or 100 mM NaCl solution (NaCl) during 4 days. Values are means of six replicates ± SE. Each replicate consists in a batch of 100 seeds.

Table 4. Germination parameters (total germination (TG %)), germination index (GI) T50 (i.e., number of days to reach 50% germination) of seeds from cvs. Libomir and Harry unprimed (UP) or primed in vermicompost leachate (1:10, v: v) (VC-priming VCP), and then sown on filter paper saturated with water (H_2O) or 100 mM NaCl (NaCl). Values are means of six replicates ± SE and different letters indicate significant differences between different treatments according to Student-Newman-Keuls test at $P < 0.05$.

	Libomir			Harry		
Cultivar	TG (%)	GI	T50 (d)	TG (%)	GI	T50 (d)
UP_{H20}	35.67 ± 1.67 c	10.71 ± 0.42 c	4.70 ± 0.28 b	93.33 ± 2.39 b	47.33 ± 1.44 c	1.58 ± a
UP_{NaCl}	19.67 ± 1.96 d	6.22 ± 0.65 c	8.08 ± 0.54 a	93.67 ± 1.14 b	37.35 ± 2.12 d	2.24 ± b
VCP_{H20}	95.33 ± 1.76 a	68.47 ± 4.43 a	1.03 ± 0.28 c	100.00 ± 0.00 a	88.17 ± 0.50 a	<1
VCP_{NaCl}	87.33 ± 2.91 b	55.19 ± 5.20 b	1.42 ± 0.11 c	99.33 ± 0.67 a	73.06 ± 2.41 b	<1

Table 5. Fresh and dry weight (mg/plant) and length (cm/plant) of seedling grown from unprimed (UP) and primed seeds of cvs. Libomir and Harry 7days on water (H_2O) or 100 mM NaCl (NaCl).The seeds were primed in vermicompost leachate (1:10, v: v) (VC-priming VCP). Values are means of six replicates ± SE. Different letters indicate significant differences between different treatments according to Student-Newman-Keuls test at $P < 0.05$.

	Libomir			Harry		
Cultivar	FW (mg)	DW (mg)	L (cm)	FW (mg)	DW (mg)	L (cm)
UP_{H20}	58.49 ± 6.69 a	5.63 ± 1.09 a	7.73 ± 0.69 ab	61.53 ± 5.67 a	3.09 ± 0.36 ab	9.75 ± 0.49 b
UP_{NaCl}	64.03 ± 0.27 a	3.29 ± 0.16 a	5.01 ± 0.40 b	40.86 ± 7.33 ab	3.03 ± 0.10 ab	6.95 ± 0.47c
VCP_{H20}	29.27 ± 0.38 b	2.32 ± 0.39 a	9.03 ± 0.79 a	58.18 ± 2.72 a	3.74 ± 0.26 a	11.20 ± 0.21a
VCP_{NaCl}	35.56 ± 0.24 b	3.01 ± 0.24 a	9.13 ± 0.60 a	30.77 ± 4.90 b	2.01 ± 0.15 b	9.57 ± 0.12b

Sodium accumulated in seedlings germinating from unprimed seeds in the presence of NaCl (Figure 4(a)). Seedlings issued from primed seeds germinating in water did not contain a higher Na^+ content than those obtained from unprimed ones, but seedlings issued from primed seeds and germinating in the presence of NaCl exhibited higher Na^+ content than those issued from unprimed seeds for both cv. Salinity reduced K^+ content in seedlings issued from unprimed seeds of cv. Libomir. Priming with VCL also lead to a decrease in K^+ content in seedlings of Libomir germinating in water and in salt conditions. Seedlings from cv. Harry exhibited a constant K^+ value (Figure 4(b)).

Seeds primed with VCL were analyzed for proline and MDA content at the end of dehydration following priming (see Material and Methods) and compared with dry unprimed seeds. As shown in Figure 4(c), primed seeds from both cultivars contained higher proline than unprimed one while lower MDA concentration was observed for cv. Harry.

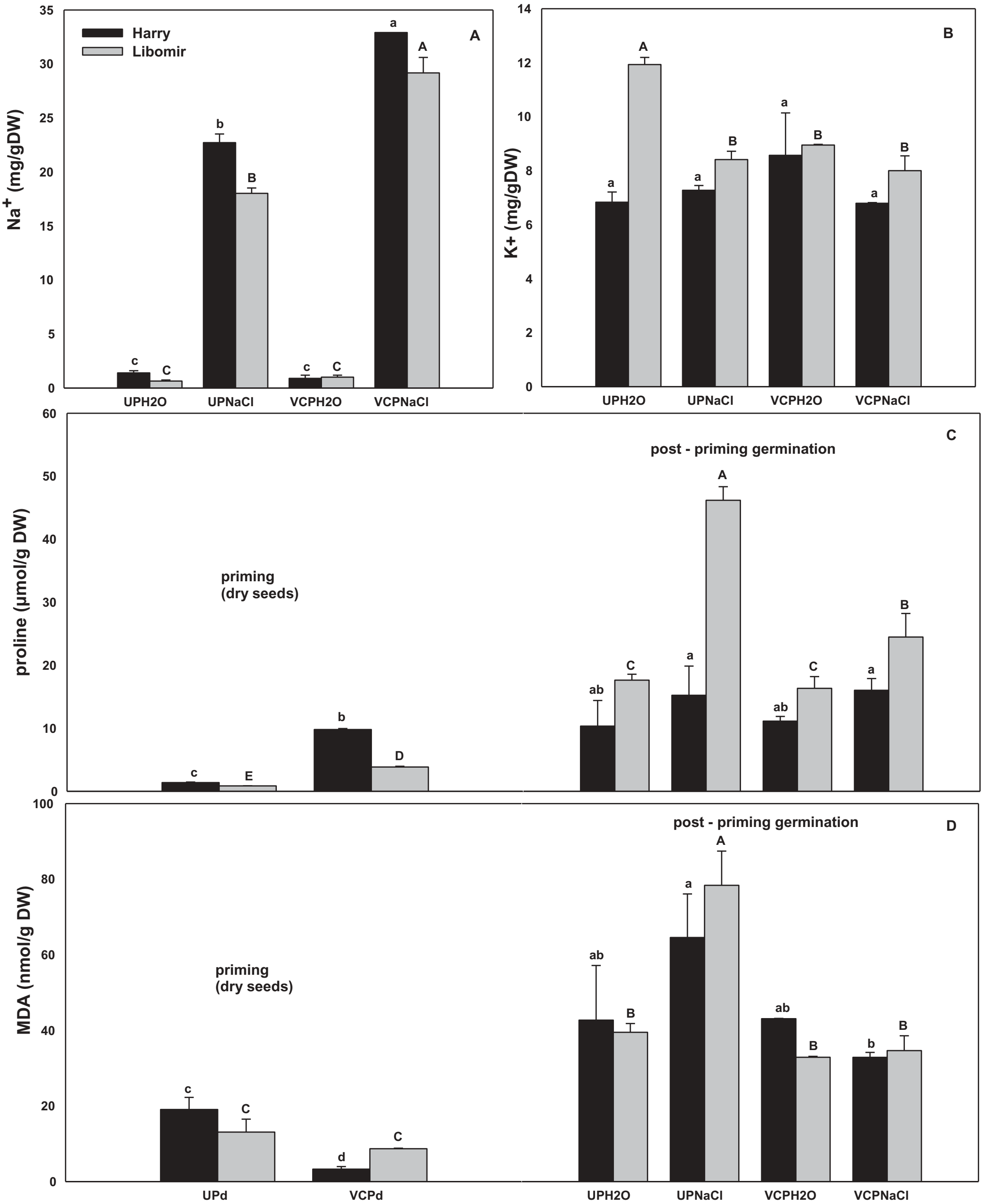

Figure 4. Sodium (a), potassium (b), proline (c) and malondialdehyde MDA (d) concentration of seedlings of *Brassica napus* cvs. Libomir and Harry grown from unprimed (UP) and VCL-primed (P) seeds of Harry and Libomir maintained during 7 days on water (control) or 100 mM NaCl. Values are means of three replicates ± SE and different letters indicate significant differences between different treatments according to Student-Newman-Keuls test at $P < 0.05$. For proline and MDA, data are also provided for dry unprimed seeds (UPd) and dried primed ones (VCPd).

During subsequent germination, proline concentration (Figure 4(d)) strongly increased in seedlings issued from unprimed seeds of cv Libomir germinating in the presence of NaCl, but the recorded increase was lower for seedlings issued from primed seeds. Proline concentration remained constant in seedlings of cv. Harry. The MDA concentration increased in NaCl-treated seedlings of cv. Libomir issued from unprimed seeds but was not affected in primed seeds. Similarly, the MDA concentration decreased in NaCl-treated seedling of cv. Harry issued from primed seeds comparatively to those obtained from unprimed ones.

Discussion

Improving seed germination under salt-stress conditions is an important challenge for agronomists, plant breeders, and farmers. As a natural by-product of organic matter digestion by earthworms, vermicompost leachate possesses interesting properties for this purpose. The present study demonstrates that it is especially efficient when used as a priming agent to allow subsequent germination and early seedling growth in the presence of NaCl, and that its effects vary with the cultivar and/or seed age. Moreover, vermicompost leachate also appears as an interesting rejuvenation compound able to limit the deleterious impact of aging in old seed lots.

Vermicompost leachate was shown to improve seed germination in numerous plant species (Arancon et al. 2008; Ievinsh 2011; Kandari, Kulkarni, and Van Staden 2011; Rupani et al. 2018). The present study suggests that this is especially the case for aged seed lots: seeds from cv. Libomir increased their final germination percentage by more than 70% when exposed to VCL during germination.

Seed aging is often considered to result from the accumulation of damages caused by free radicals among which H_2O_2 is considered as the most critical agent because of its stability at biological pH and ability to cross membranes (Kibinza et al. 2011). This may especially be the case in oil-rich seeds stored under non-optimal conditions (Hsu et al. 2003). Malondialdehyde was frequently reported as a reliable index of oxidative stress encountered by plant tissues. In the present study, however, the MDA concentration of unprimed seeds was similar in both cultivars (Figure 4) and the lower germination percentage of cv. Libomir could thus hardly be explained by the free radical theory. Other biochemical and structural changes may also occur as a result of seed aging, but our work suggests that VCL may contribute to repairing processes if accumulated damages are not irreversible. In contrast, VCL had only a minor impact on cv. Harry probably because this seed lot was intact and already presented an optimal germination under control conditions.

Vermicompost leachate modified young seedling elongation under control conditions. Although it did not modify seedling weight, VCL clearly increased hypocotyl length. A similar effect was already reported by Arancon et al. (2012) and was attributed the presence of phytohormones in VCL. Kanto et al. (2015) reported that rice seedlings issued from artificially aged seeds exhibited an altered elongation; seedlings from cv. Libomir were indeed smaller than seedlings from cv. Harry. The VCL used in the present study contains both auxins and cytokinins (Table 1). Gibberellins are also known to hasten seed germination and cell elongation and were reported to have a positive impact on rapeseed germination (Benincasa et al. 2013). However, gibberellins remained below the level of detection in our VCL. This contrasts with the data reported by Arancon et al. (2012) and by Singh, Kulkarni, and Van Staden (2014) who reported the presence of gibberellins and GA-like activity in VCL. This also illustrates the fact that VCL composition is not standard and strongly varies according to the source of organic matter and composting conditions (Aremu et al. 2015; Ibrahim, Quaik, and Ismail 2016; Rupani et al. 2018; Tejada et al. 2008).

The advantage afforded by VCL when added to NaCl-containing solution was not obvious. Indeed, VCL had only a limited impact on TG values and even increased T50 comparatively to NaCl treatment alone. Sodium slightly accumulated in VCL-treated seedlings of cv. Harry. Vermicompost leachate itself contains Na^+ concentration (Table 1) at a concentration of 40 mM. However, VCL was diluted 10 times in the present study and the additional presence of Na^+ did not lead to higher Na^+ content in the seeds exposed to NaCl. Salinity also induced K^+ decrease in cv. Libomir while VCL which contains potassium increased K^+ content in both genotypes although this was not sufficient to increase the germination percentage, suggesting that K^+ deficiency was not responsible for salt-induced decrease in seed germination capacity. Proline is also assumed to play key role in NaCl tolerance (Munns 2002; Munns and Tester 2008) but this compound was not detected in our VCL (Benazzouk and Lutts, unpublished data). However, salinity increased proline content in cv. Libomir only and this synthesis was even increased by the additional presence of VCL with no clear impact on final germination suggesting that increased proline remained insufficient to provide additional salinity resistance.

It is noteworthy that the positive impact of VCL on salt tolerance at the germination phase was by far higher when VCL was used as a priming agent than added directly to the germination medium. In Libomir seeds primed with VCL and then germinating in the presence of NaCl, TG and GI values were 87.33% and 55.19, respectively (Table 4). In contrast, when VCL was directly added to NaCl-containing germinating solution for unprimed seeds, corresponding values were only 26.67% and 5.47, respectively (Table 2). Benazzouk, Djazouli, and Lutts (2018) recently reported that VCL applied before salt stress on young tomato plants was more efficient for avoidance of subsequent NaCl constraint than VCL applied concomitantly to salinity. The present study suggests a similar picture at the germination phase and reinforces the hypothesis that the metabolic impact of VCL requires enough time to fully operate.

The present study focuses on two precise rapeseed cultivars. However, an additional set of experiments was performed with old seed lots from eight other cultivars and provided similar trends (supplemental data, Table S1): in all cases, VCL priming increased the germination percentages in the absence of salt and, in several cases, also increased TG in seeds germinating in the presence of 100 mM NaCl. A positive impact of VCL priming on the kinetics of germination was also confirmed by a significant increase in GI and a decrease in T50.

Kubala et al. (2015a) reported that priming in rapeseed is a complex molecular process and that all phases of priming, including soaking, drying and post-priming germination trigger-specific genes expression and protein synthesis. According to these authors, changes in gene expression may even be influenced by epigenetic changes such as DNA methylation while Marconi et al. (2013) demonstrated that salinity itself occurring after priming may also induce DNA methylation in rapeseed. According to Kubala et al. (2015b) priming in *Brassica napus* triggers the expression of genes involved in proline synthesis (*P5CSA* coding for pyrroline-5-carboxylate synthetase) and inhibits gene coding for proline dehydrogenase involved in proline degradation. Dehydration occurring during priming after the soaking phase may be considered as a stressing phase hardening the seed material for subsequent germination in salt-containing solution. Consequently, proline was higher in primed than unprimed seeds for both cultivars (Figure 4(c)). However, further increase in proline during subsequent germination once again only occurred in the aged seed lot of cv. Libomir and not in cv. Harry, which is on line with the data of Kanto et al. (2015) who reported that a given priming treatment may have distinct effects on a same parameter depending on the age of the concerned seed lot. Singh, Kulkarni, and Van Staden (2014) reported that VCL treatment reduced proline concentration in *Phaseolus vulgaris*. It has to be noted that primed seeds of Libomir germinating in the presence of NaCl presented lower proline content but a higher germination percentage than unprimed ones, suggesting, once again, that a high proline content is not a pre-request for germination in the presence of NaCl.

Some compounds present in VCL were reported to act as efficient seed priming agent. This is the case for humic acids which strongly influence gene expression involved in gibberellins and abscisic acid metabolism (Sheteiwy et al. 2017). Phenolic compounds acting as antioxidant may also be of primary importance for germination in the presence of NaCl and MDA content was indeed decreased in primed seeds comparatively to unprimed ones (Figure 4(d)). Kibinza et al. (2011) demonstrated in sunflower that catalase involved in H_2O_2 detoxification is a key enzyme in seed recovery from aging during priming.

Efficient seed priming by phytohormones has also been reported (Jisha and Puthur 2016; Lutts et al. 2016; Zhang et al. 2007). Polyamines are key molecules assuming a wide range of protective functions at all phases of plant development and the three major polyamines (putrescine, spermidine, spermine) were reported to be present in VCL (Table 1). Afzal et al. (2009) provided clear evidences that polyamines priming increases tomato seed vigor and germinating capacity. The presence of cytokinins acting as antisenescent plant hormones may also afford advantages to seeds and young seedlings exposed to NaCl (Zhang et al. 2014). It can thus not been excluded that the obvious efficiency of VCL as a seed priming agent for salinity resistance may be the ultimate consequences of the simultaneous presence of several interacting compounds rather than the result of one single molecule.

Conclusions

Vermicompost leachate is a promising compound to improve germination in seeds of *Brassica napus* exposed to salinity. When used as a priming agent, it improves the final germination percentage and germination index but its effects depend on the considered cultivar and age of the seeds. Vermicompost leachate was especially efficient for rejuvenation of old seed lots in relation to an improved management of the oxidative stress. Positive effect of vermicompost might be related to its content in antioxidant and phytohormones. It thus appears as a useful eco-friendly product to improve germination of old seed lots in salt-affected soils.

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