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Full Length Research Paper

Effect of saturation on seed dormancy and germination of the halophyte *Leymus chinensis*

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The halophyte, *Leymus chinensis* (Trin.) a perennial rhizomatous grass, is widely distributed in northern China, Mongolia and Siberia. Due to its ecological and economical significance, understanding the underlying causes of the low fecundity and long-term seed dormancy that are characteristic of *L. chinensis* is critical for promoting propagation and germination of this grass. This study investigates the effect of saturation treatments of mature seeds on breaking seed dormancy and increasing germination in *L. chinensis*. The germination rates were significantly increased to 33.81, 46.15 and 50.35% from LcWT07-1, LcWT07-2 and LcJS0107, respectively after saturation treatments at 4°C for 3 days, suggesting that germination inhibitor components had been lixiviated from seed coats and/or mature seeds. High-performance liquid chromatography (HPLC) and ultraviolet (UV) analysis demonstrated that acidic components were lixiviated from both seed coats and dehusked mature seeds. Since dehusking also promotes seed germination, we predicted that the seed coat-derived soluble components contained germination inhibitors. Indeed, when *L. chinensis* plantlets were watered with the lixiviated solutions, their germination, but not their growth and development, was inhibited. Taken together, this work provides valuable insight into the regulation of seed dormancy and germination rate in *L. chinensis*, which may in turn have implications for improved propagation.

Key words: Saturation treatment, HPLC, UV, seed dormancy, germination, *Leymus chinensis*.

INTRODUCTION

The halophyte *Leymus chinensis* (Trin.), a perennial rhizomatous grass belonging to the tribe Poaceae (Czerepanov, 2007), is a constructive species widely distributed in the meadow steppe of the eastern Eurasian grasslands and the Loess Plateau, Songnen Plain and Nei Mongol Plateau in China (Liu et al., 2002). Due to its intrinsic adaptation to highly alkali-sodic soil conditions (Anamthawat Jónsson et al., 1990; Jin et al., 2006), *L. chinensis* has been used as a soil-binding plant to protect soil from desertification in the arid areas of northwest China. This environmental adaptability, combined with fine agronomic properties such as rich productivity, high protein content, adaptability to its surroundings and palatability to cattle, has made *L. chinensis* a useful component of artificial grasslands constructed to improve ecological conditions in Western China (Jia, 1987). However, despite these attributes, *L. chinensis* exhibits low sexual reproductivity, and its slow propagation cannot compensate for the destructive effects of deteriorating environmental conditions, human activity and extensive grazing (Wang et al., 2005). Major causes of *L. chinensis* seed germination inhibition include protandry in *L. chinensis*, which limits pollination within flowering shoots and results in self-incompatibility (Wang et al., 2005;

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Abbreviations: **HPLC,** High-performance liquid chromatography; **UV,** ultraviolet; **ABA,** abscisic acid; **PEG,** polyethylene glycol; **GA_3,** gibberellic acid; **6-BA,** 6-benzyladenine; **MS,** Murashige and Skoog; **ANOVA,** analysis of variance.

Table 1. Basic characteristics of LcWT07-1, LcWT07-2 and LcJS0107 mature seeds.

Plant genotype	LcWT07-1	LcWT07-2	LcJS0107
Collection year	2007	2007	2007
Voucher collection	Siping, Jilin, China	Siping, Jilin, China	Changchun, Jilin, China
Dry mature seed number (g^{-1})	425.5 ± 5.5[a]	402.2 ± 10.2[a]	427.8 ± 8.4[a]
Practical mature seed number (g^{-1})[z]	222.6 ± 3.0[a]	266.4 ± 5.6[a]	366.8 ± 4.5[a]
One dry seed weight (mg)	2.35 ± 0.05[a]	2.49 ± 0.02[a]	2.34 ± 0.08[a]
One mature seed weight (mg)	1.25 ± 0.02[a]	1.22 ± 0.01[a]	1.13 ± 0.02[a]
Barren seed occupancy rate (%)	47.76[a]	33.83[ab]	14.05[b]
Mature seed occupancy rate (%)	52.24[a]	66.17[a]	85.95[a]
Weight per 1000 mature seeds with seed coats (g)	2.35 ± 0.10[a]	2.49 ± 0.05[a]	2.34 ± 0.05[a]
Weight per 1000 de-husked mature seeds (g)	1.25 ± 0.02[a]	1.22 ± 0.01[a]	1.13 ± 0.02[a]
Weight ratio of seeds to seed coats (%)[y]	38.56 ± 0.8[a]	47.91 ± 1.0[a]	71.38 ± 0.6[a]

Means followed by the same lowercase letter in the same line are not significantly different at $P < 0.05$ according to two-way ANOVA using Duncan's multiple-range test. [z]Dehusked mature seed number is the coated mature seed number minus barren seed number. [y]Ratio of seed to seed coat weight is the ratio of dehusked mature seed weight to seed coat weight per gram of coated mature seeds, with a higher weight ratio indicating a higher degree of maturity.

Huang et al., 2004), and seed dormancy (Ma et al., 2008).

The germination of halophytes is controlled by several environmental factors, including light (Huang and Gutterman, 1998), temperature (Badger and Ungar, 1989), and salinity (Ungar, 1995). Seed dormancy is also influenced by these environmental factors (Holdsworth et al., 2008), as well as plant hormones (Bewley, 1997; Carrera et al., 2008) and several molecular signaling pathways (Bethke et al., 2004; Sarath et al., 2007). The seed dormancy style of *L. chinensis* has been characterized as inhibitor-induced physiological dormancy, with abscisic acid (ABA) being one of the key inhibitors (Ma et al., 2005). Previous physical and chemical approaches to break seed dormancy in *L*. chinensis have included seed coat removal, pre-cold treatment and variable temperature, polyethylene glycol (PEG) treatment, salt stress treatment and exogenous gibberellic acid (GA_3) or 6-benzyladenine (6-BA) treatment (Yi, 1994; Yi and Zhang, 1995; Ma et al., 2005; Zhou and Yang, 2004; Ma and Liang, 2007). However, these methods have not shown a clear effect on increasing germination rate.

In this study, we saturated three mature seed samples, including two ecotypes, with 4°C sterile water to lixiviate the predicted germination inhibitors. We analyzed the soluble extracts lixiviated from coated mature seeds, dehusked mature seeds and seed coats by high-performance liquid chromatography (HPLC) and ultra-violet (UV) analysis. To detect whether the lixiviated components could promote the maintenance of seed dormancy and/or inhibition of seed germination, we examined the germination rates of mature seeds after saturation, germination rates of dehusked mature seeds and plant growth responses following exogenous application of lixiviated solutions. Our results suggested that saturation in sterile water can efficiently break seed dormancy and thus, increase germination rates. Furthermore, the lixiviated solutions specifically inhibited seed germination but not seedling growth or development.

MATERIALS AND METHODS

Mature seeds of wild-type genotype, LcWT07-1 and LcWT07-2 strains were obtained from the natural grasslands in Siping, Jilin, China. Mature seeds of LcJS0107, a newly cultivated variety, were obtained from the Jisheng Chinese Wildrye Excellent Seed Station, Changchun, Jilin, China (Sun and Hong, 2011). The mature seeds were naturally air-dried and stored at 4°C. Coated and dehusked mature seeds were both surface-sterilized (Sun and Hong, 2010), with 70% ethanol for 1 min followed by 5% sodium hypochlorite for 20 min. The surface-sterilized mature seeds were then washed 5 times with an excess of sterile water.

Saturation treatment

1 g of coated mature seeds was put in 500 ml tissue culture pots with 100 ml sterile water for extracting the soluble components at 4°C. To compare the changes in the lixiviated components, the dehusked mature seeds and seed coats from 1 g of coated mature seeds (dehusked mature seed/seed coat weight ratio, shown in Table 1) were separately incubated in 100 ml sterile water for extracting at 4°C. The lixiviated solutions were collected and replaced by 100 ml fresh sterile water every 24 h for 3 days.

High-performance liquid chromatography (HPLC) analysis of soluble components

The lixiviated solutions obtained following saturation were analyzed by an HPLC system (CBM-20A, Shimadzu Co. Ltd., Japan) with two gradient pump systems (LC-20AT, Shimadzu), a UV-detector (SPD-10A, Shimadzu), an auto sample injector (SIL-20A, Shimadzu) and a column oven (CTO-20A, Shimadzu). A prevailing C18 column (4 µm, 150 × 4.6 mm, Synergi Fusion-RP 80A, USA) was used. The flow rate of mobile phase solution was 1.0 ml/min. The mobile phase solution was run by a gradient system as follows: solution A (0.4%, v/v, formic acid in distilled deionized water) and solution B (acetonitrile), with a gradient elution programmed to 2 to 5% of

solution B for 0 to 10 min, 5 to 15% of solution B for 10 to 20 min, 15 to 30% of solution B for 20 to 30 min and 30 to 100% of solution B for 30 to 50 min. Sample injection volume was 10 µL. Peaks were monitored at 280 nm. All lixiviated solutions were condensed and redissolved in 10 ml distilled deionized water for sample injection.

Ultraviolet (UV) spectrophotometry analysis of lixiviated components

Wave scan from 200 to 900 nm was performed on the lixiviated solutions using UV spectrophotometry (NanoPhotometer^{TM}, IMPLEN, UK). The absorbance at specific wavelengths was measured using Single Wavelength program of UV spectrophotometry.

Inhibition assay

The lixiviated solutions were added into culture medium (1:100, v/v) to evaluate the effect on the germination rates of dehusked mature seeds. The culture medium used for germination contained Murashige and Skoog (MS; Murashige and Skoog, 1962) basic salts, 30 g L^{-1} sucrose and 4.0 g L^{-1} gelrite for solidification. The pH of the medium was adjusted to 5.8 prior to the addition of gelrite. The culture medium was then sterilized by autoclaving at 120°C and 1.4 kg cm^{-2} for 20 min. Dehusked mature seeds (n = 30) were cultured in this culture medium in disks (90 mm in diameter × 15 mm in height) at a density of 10 seeds per disk. The cultures were stored at 25°C, with a 16/8 h (day/night) photoperiod and a relative humidity of 45 to 70%. The germination rates were examined and calculated by the ratio of germinated seed number to total sown seed number after 15 days culture. Three independent repetitions were performed for each germination rate experiment.

The saturation extraction solutions from different saturation times were also used for watering one-week-old LcJS0107 seedlings (n = 3) to evaluate the effect on plant growth and development. The surface-sterilized coated mature seeds were germinated on sterile filter paper wetted with sterile water at 25°C, and the seedlings were grown in pots (20 cm in diameter × 20 cm in height) containing clay/vermiculite (3/1, v/v) at a density of one seedling per pot. The plants were grown at 25°C in greenhouse conditions using a 16/8 h (day/night) photoperiod and a relative humidity of 45 to 70%. During the treatment period, plant height, leaf number, leaf length and width and thickness of the main daughter rhizome plant were measured. Three independent repetitions were performed for each separate analysis on growth and development.

Statistical analysis

Differences between the means were determined by two-way analysis of variance (ANOVA) using Duncan's multiple-range test (Duncan, 1955). A *P* value of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Morphological characteristics of LcWT07-1, LcWT07-2 and LcJS0107

Mature seeds of LcWT07-1 and LcWT07-2 strains were collected from natural grasslands in Jilin, China, while mature seeds of LcJS0107 were collected from artificial planting fields in Jilin, China. There were more adulterants, such as spike haulms in LcWT07-1 and LcWT07-2 mature seed samples, compared with LcJS0107 (Figure 1); however, there were no significant differences in mature seed number per gram or thousand-seed weight between the three genotypes (Table 1). The dehusked mature seed number per gram of LcJS0107 was relatively higher than that of LcWT07-1 and LcWT07-2, and the LcJS0107 mature seed occupancy rate of 85.95% represented the highest rate among that of the three genotypes. Furthermore, the barren seed occupancy rate of LcJS0107 was significantly lower than that of LcWT07-1, and LcJS0107 displayed the highest ratio of seed to seed coat weight. Taken together, the LcJS0107 mature seed sample used in this study exhibited a higher maturation level than the other two genotypes.

Germination rates of LcWT07-1, LcWT07-2 and LcJS0107

Ma and Liang (2007) reported that the germination rates of mature *L. chinensis* seeds are closely linked to the environmental conditions. However, even in environmental stressed conditions, 4- to 5-year-old mature seeds have the highest germination rates and are capable of self-germination (Ma et al., 2005). Using these criteria, the 3-year-old mature seeds used in this study are still in their seed dormancy period. This was validated by our observation that the germination rates of the mature seeds ranged from only 3 to 10% when grown in normal conditions (Table 2). Among the three mature seed genotypes, the germination rate of LcJS0107 was significantly higher ($P < 0.05$) than the rates observed for either LcWT07-1 or LcWT07-2. Moreover, it has been reported that germination commences with the uptake of water by the quiescent seed and terminates with the elongation of the embryonic axis (Bewley and Black, 1994; Holdsworth et al., 2008). Liang and Ma (2006) have suggested that the seed coats wrapping *L. chinensis* mature seeds promote seed dormancy and depress the germination rate due to decreased ventilation and retention of components that inhibit germination. We therefore hypothesized that dehusking the mature seeds would increase the germination rate. After dehusking, the germination rates of LcWT07-1, LcWT07-2 and LcJS0107 were increased by 3.47-, 1.88- and 0.96-fold, respectively relative to those of coated mature seeds (Table 2).

Saturation treatment for 1 day increased the germination rates of both dehusked and coated mature seeds, although the dehusked mature seeds maintained their higher germination rates in all three genotypes. For coated mature seeds, the germination rates gradually increased over the saturation time course, reaching the highest levels after a 3-day saturation treatment. For dehusked mature seeds, the germination rates significantly increased after 1 day of saturation treatment and

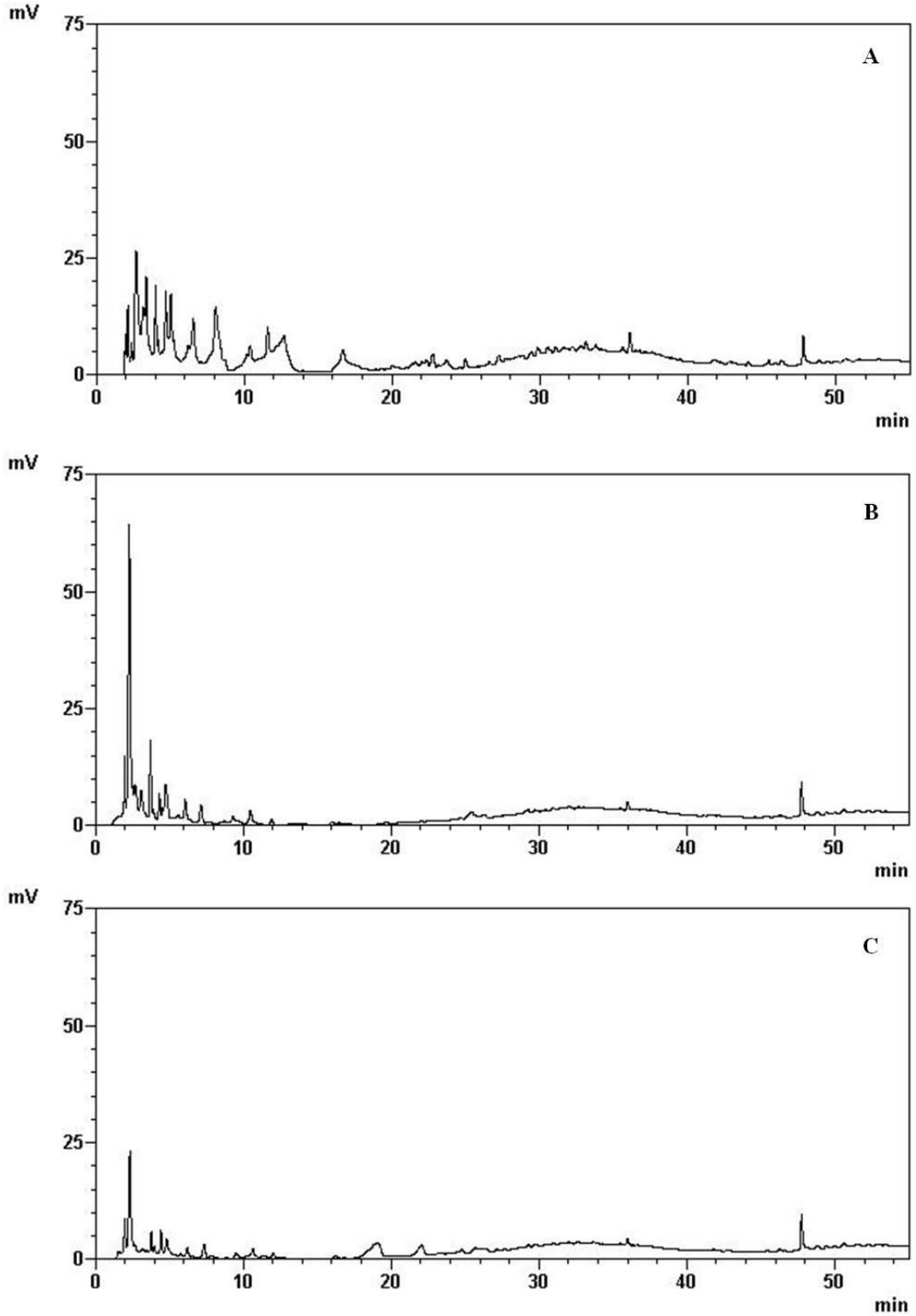

Figure 1. HPLC analysis of solutions lixiviated from coated mature seeds of LcWT07-1 (A), LcWT07-2 (B) and LcJS0107 (C).

reached a maximum after 2 days. Although, the germination rates in dehusked seeds began to decline by 3 days, the rates of these seeds were still significantly higher than those that did not undergo saturation

Table 2. Germination rates of dehusked and coated mature seeds with various saturation periods (day 0, 1, 2 and 3).

Plant genotype		Saturation period (day)			
		0	1	2	3
LcWT07-1	Non de-husked mature seeds	2.82 ± 0.24^{b}	11.48 ± 0.30^{a}	28.16 ± 0.16^{a}	33.81 ± 0.32^{a}
LcWT07-2		4.98 ± 0.20^{b}	8.81 ± 0.16^{a}	30.61 ± 0.25^{a}	46.15 ± 0.12^{a}
LcJS0107		9.84 ± 0.24^{b}*	16.99 ± 0.14^{ab}	35.35 ± 0.22^{a}	50.35 ± 0.20^{a}
LcWT07-1	De-husked mature seeds	12.61 ± 0.20^{b}	24.24 ± 0.12^{a}	36.35 ± 0.20^{a}	29.33 ± 0.18^{a}
LcWT07-2		14.34 ± 0.26^{b}	27.68 ± 0.30^{ab}	48.06 ± 0.28^{a}	36.54 ± 0.36^{a}
LcJS0107		19.23 ± 0.30^{b}	39.35 ± 0.16^{ab}	52.09 ± 0.28^{a}	42.35 ± 0.32^{a}

Germination rates are the mean of three repeated experiments. Means followed by the same lowercase in the same line are not significantly different while an asterisk (*) indicates a significant difference between the germination rates of different plant genotypes with the same saturation period at $P < 0.05$, according to two-way ANOVA using Duncan's multiple-range test.

treatment.

UV analysis of different saturated solutions

To detect the predicted germination inhibitors in lixiviated solutions, a wavelength scan (200 to 990 nm) was performed using UV analysis. There were many absorbency peaks at specific wavelengths, with the absorbances shown in Tables 3 and 4. Lixiviated solutions derived from seed coats and dehusked mature seeds showed absorbency peaks at 251, 265, 275, 287, 292, 850, 882, 890, 916 and 936 nm, with the highest peaks at 265, 275, 287 and 292 nm. The lixiviation rate and solution composition were influenced by the saturated component (coated mature seed, dehusked mature seed or seed coat), sample type and total volume of the soluble extract. For example, in lixiviated solutions derived from coated mature seeds, the absorbency peaks at 251, 850, 882, 890 and 916 nm were nearly undetectable.

The absorbance of most components at their specific wavelengths decreased over the course of saturation. However, some components had a higher absorbance after a 3-day saturation treatment (Tables 3 and 4). Generally, 1 day of saturation treatment could lixiviate 43.7 to 78.9% of the total extracted material. The component contents in the lixiviated solutions from day 2 showed 36.1 to 88.1% decreases compared with day 1, with the exception of the component represented by the 265 nm peak in LcWT07-2 seeds, which remained unchanged. After a 3-day saturation treatment, the absorbance of saturated components generally declined rapidly, with several exceptions. However, the 3-day-treated lixiviated components from LcWT07-2 seed coats measured at 287, 292 and 275 nm, were 265.5, 242.2 and 244.5% higher, respectively than the 2nd day lixiviated solutions of seed coats. Similarly, at the 3rd day of treatment, lixiviated components from LcWT07-1 coated mature seeds at 287, 292 and 265 nm, were increased by 25.8, 17.8 and 58.3%, respectively compared with the 2nd day lixiviated solution from coated mature seeds. Similar results were found for lixiviated components from coated mature seeds of LcWT07-1 and LcWT07-2 measured at 265 nm, such that the absorbance in the 3-day-treated lixiviated components from coated mature seeds of LcWT07-1 and LcWT07-2 showed 33.2 and 5.2% increments, respectively, compared with the 2nd day lixiviated solution from coated mature seeds.

According to the absorbance readings at 251, 850, 890 and 916 nm in lixiviated solutions from dehusked mature seeds and seed coats, there were few changes as a result of the saturation treatment. However, the components with absorbance at 936 and 916 nm were nearly nonexistent in lixiviated solutions from dehusked mature seeds, suggesting that these two components exist primarily in seed coats and are lixiviated prior to day 3 of saturation treatment.

HPLC analysis of lixiviated solutions

To further characterize the predicted germination inhibitors in lixiviated solutions, HPLC analysis was done with a C18 column with detection at 280 nm. The solutions lixiviated from LcWT07-1, LcWT07-2 and LcJS0107 coated mature seeds contained 31, 34 and 27 discrete components (as measured by peak area percentages exceeding 1%), respectively comprising 25.83, 32.08 and 33.33% of total peak numbers (Table 5 and Figure 1). The LcWT07-1 genotype was distinguished by 14 unique components, as compared with 6 unique components in the LcWT07-2 and 4 in the LcJS0107. There were at least 18 common components appearing in peaks at the same retention time (Ret. Time) (Table 6 and Figure 1). The highest peak in the LcWT07-1 appeared at a Ret. Time of 2.687 min, with 5.852% of total area; however, in the LcWT07-2 and LcJS0107, the highest peak appeared at a Ret. Time of about 2.3 min, with 14.699 and 16.730% of relevant total area, respectively (Table 6).

Table 3. UV analysis of different solutions lixiviated from seed coats and dehusked mature seeds.

Saturation sample	Ecotypes	Saturation period (day)	Wavelength (nm)									
			287	292	265	275	936	251	850	890	882	916
Seed coats	LcWT07-1	1	1.685	1.760	0.580	0.619	0.030	0.010	0.016	0.012	0.011	0.017
		2	0.641	0.616	0.474	0.395	0.021	0.003	0.018	0.013	0.008	0.018
		3	0.143	0.156	0.158	0.104	0.003	0.005	0.009	0.006	0.001	0.011
	LcWT07-2	1	1.229	1.222	0.486	0.474	0.032	0.000	0.020	0.011	0.012	0.019
		2	0.362	0.357	0.310	0.256	0.008	0.003	0.011	0.006	0.002	0.012
		3	1.182	1.221	0.143	0.883	0.742	0.006	0.011	0.005	0.001	0.012
	LcJS0107	1	1.068	1.061	0.342	0.527	0.041	0.004	0.030	0.018	0.017	0.026
		2	0.258	0.261	0.171	0.200	0.007	0.003	0.009	0.007	0.000	0.010
		3	0.097	0.108	0.138	0.094	0.004	0.005	0.011	0.003	0.000	0.008
Seeds	LcWT07-1	1	0.173	0.192	0.204	0.157	0.009	0.004	0.014	0.007	0.003	0.013
		2	0.021	0.036	0.038	0.026	0.001	0.005	0.005	0.005	0.000	0.008
		3	0.026	0.042	0.061	0.016	0.000	0.002	0.008	0.004	0.000	0.010
	LcWT07-2	1	0.066	0.090	0.109	0.064	0.000	0.005	0.007	0.003	0.000	0.009
		2	0.040	0.049	0.109	0.040	0.003	0.003	0.009	0.004	0.000	0.006
		3	0.001	0.025	0.002	0.017	0.001	0.002	0.008	0.004	0.000	0.010
	LcJS0107	1	0.177	0.198	0.173	0.146	0.009	0.006	0.011	0.008	0.004	0.015
		2	0.048	0.057	0.102	0.050	0.001	0.004	0.003	0.005	0.000	0.007
		3	0.002	0.020	0.018	0.007	0.000	0.005	0.008	0.003	0.000	0.009

The seed coats or dehusked mature seeds derived from 1 g of coated mature seeds of each plant ecotype were saturated with 100 ml sterile water for the saturation periods indicated. The absorbance was the mean of three repeats.

The lixiviated solutions from LcWT07-2 coated mature seeds, dehusked mature seeds, and seed coats were also analyzed by HPLC using a C18 column with detection at the same wavelength, 280 nm. In this experiment, only 16 peaks were identified (Table 7 and Figure 2), which was a much smaller amount than in the previous experiment (Table 5 and Figure 1) due to the different concentration of the lixiviated solutions. There were at least 8 relatively abundant components appearing in all lixiviated solutions (Table 8), ranging from 14.68% of total peaks and 68.65% of total peak areas. There were at least 4 components present only in dehusked seeds, but not in seed coats, with a Ret. Time of 1.9, 2.0, 4.2 and 25.8 min, while there were other at least 3 components present only in seed coats, but not in dehusked mature seeds, with a 3.9, 4.7 and 5.3 min Ret. Time. These results suggested that there were some mutual components that could be lixiviated from both seed coats and seeds, while there were other components that could only be lixiviated from either seed coats or seeds. However, it should be noted here that there might be more mutual components in this experiment that had been erroneously observed in only a subset of the lixiviated solutions due to difficult

Table 4. UV analysis of solutions lixiviated from coated mature seeds.

Saturation sample	Ecotypes	Saturation period (day)	Wavelength (nm)				
			287	292	265	275	936
Dry mature seeds with seed coats	LcWT07-1	1	0.686	0.650	0.625	0.416	0.003
		2	0.178	0.181	0.129	0.150	0.000
		3	0.113	0.118	0.172	0.104	0.002
	LcWT07-2	1	0.452	0.442	0.323	0.270	0.014
		2	0.128	0.139	0.121	0.103	0.001
		3	0.082	0.091	0.127	0.087	0.004
	LcJS0107	1	0.241	0.245	0.283	0.183	0.000
		2	0.121	0.130	0.160	0.113	0.002
		3	0.061	0.071	0.149	0.055	0.000

One gram of coated mature seeds of each plant ecotype was saturated with 200 ml sterile water for various the saturation periods indicated. The absorbance was the mean of three repeats.

Table 5. HPLC analysis summaries of solutions lixiviated from coated mature seeds

Ecotypes	LcWT07-1	LcWT07-2	LcJS0107
Total peak No.	120	106	81
Total peak area	7920570	4426340	1700939
Peak No.	31	34	27
Peak percentage (%)	25.83	32.08	33.33
Peak area	5949278	3215666	1352487
Peak area percentage (%)	75.11	72.26	79.52

detection.

Effect of lixiviated solution treatment on germination, plant growth and development

To evaluate the effect of the lixiviated solutions on germination, plant growth and development, the germination and growth responses of one-week-old LcJS0107 seedlings watered with each solution were examined. Sterile water was used to establish baseline growth and development. No significant changes in germination rate were found after watering the seedlings with lixiviated solutions from coated mature seeds, regardless of whether mature seeds used for germination were coated or dehusked (Figure 3). Furthermore, one-week treatment with the various solutions did not elicit obvious changes in morphological characteristics or a significant effect on plant growth and development (Figure 4). A closer analysis of some growth parameters revealed that the leaf width of plants watered with the LcWT07-1 coated seed lixiviated solution was significantly higher than those watered with the LcJS0107 coated seed lixiviated solution (Figure 5), although there was no significant difference in any other plant growth parameter, such as plant height, leaf length or plant width. Additionally, after treatment with the LcWT07-1 coated seed lixiviated solution, leaf height, leaf width, rhizome number and plant width were slightly higher than the control, indicating that this solution did not inhibit, but instead slightly stimulated plant growth and development as shown in Figure 4D, F and Figure 5). Furthermore, the LcWT07-2 coated seed lixiviated solution slightly stimulated leaf width growth, while the LcJS0107 coated seed lixiviated solution slightly stimulated leaf elongation and rhizome production.

The pH value of lixiviated solutions

To understand the characteristics of the various lixiviated solutions, the pH values of these solutions, as well as the water used for saturation, were examined (Table 9). The pH value of each solution was lower compared with water, suggesting that the lixiviated components were weak-acidic. Interestingly, the pH values of nearly all solutions lixiviated from seed coats were higher than the solutions lixiviated from dehusked seeds, regardless of the length

Table 6. HPLC analysis of main peaks with retention times (Ret. Time) and areas in solutions lixiviated from coated mature seeds.

Peak number	LcWT07-1			LcWT07-2			LcJS0107		
	Ret. Time	Area	Area%	Ret. Time	Area	Area%	Ret. Time	Area	Area%
1	**1.958**	47234	0.596	**1.975**	110868	2.505	**1.972**	71995	4.233
2	**2.396**	56997	0.720	**2.296**	650646	14.699	**2.339**	284567	16.73
3	**2.687**	463531	5.852	**2.695**	129349	2.922	**2.670**	45303	2.663
4	**3.162**	206869	2.612	**3.098**	146507	3.31	**3.195**	50269	2.955
5	**4.013**	161699	2.042	3.916	43578	0.985	**3.995**	35181	2.068
6	22.025	32232	0.407	22.031	11979	0.271	**22.038**	57399	3.375
7	**27.21**	82113	1.037	**27.121**	47285	1.068	27.147	9691	0.57
8	**30.531**	86313	1.09	**30.461**	69290	1.565	**30.458**	24116	1.418
9	**31.058**	98190	1.24	**30.967**	74604	1.685	**30.968**	24736	1.454
10	**32.77**	135036	1.705	**32.689**	89979	2.033	**32.677**	30212	1.776
11	**33.111**	123553	1.56	**33.037**	64688	1.461	**33.029**	12604	0.741
12	**33.769**	112744	1.423	**33.715**	51171	1.156	**33.696**	17777	1.045
13	**34.789**	41714	0.528	**34.724**	66177	1.495	**34.675**	1840	0.108
14	**36.082**	133354	1.684	**36.002**	77598	1.753	**35.983**	13091	0.770
15	37.178	41626	0.526	**37.111**	47941	1.083	37.095	1393	0.082
16	**47.801**	63049	0.796	**47.751**	73734	1.666	**47.75**	75135	4.417
17	50.709	21711	0.274	50.66	21725	0.491	**50.654**	22230	1.307
18	**51.574**	16091	0.203	51.538	19564	0.442	**51.534**	20181	1.186
19	**4.703**	215533	2.721	**4.758**	150815	3.407			
20	**5.242**	131020	1.654	5.267	25146	0.568			
21	**7.091**	36076	0.455	**7.147**	63244	1.429			
22	**10.412**	112799	1.424	**10.491**	66126	1.494			
23	**16.669**	249731	3.153	16.675	9648	0.218			
24	**35.205**	79240	1.000	**35.214**	48995	1.107			
25	37.578	69331	0.875	**37.501**	47407	1.071			
26	**37.932**	104915	1.325	**37.876**	72487	1.638			
27	**4.406**	40125	0.507				**4.438**	56163	3.302
28	4.877	44019	0.556				**4.836**	82681	4.861
29	**6.211**	149047	1.882				**6.205**	23747	1.396
30	**6.561**	249788	3.154				6.592	2847	0.167
31	9.567	28384	0.358				**9.515**	12534	0.737
32	25.783	18209	0.23				**25.726**	21560	1.268
33				**3.727**	142941	3.229	**3.785**	46417	2.729
34				**11.926**	17283	0.390	**12.036**	13786	0.810
35				24.713	24478	0.553	**24.754**	17159	1.009
36				**26.317**	67757	1.531	**26.382**	17580	1.034
37				**29.271**	94267	2.13	**29.281**	25032	1.472
38				**31.485**	75962	1.716	**31.507**	25567	1.503

Table 6. Contd.

39				**32.04**	128459	2.902	**32.031**	30883	1.816
40				**33.419**	45349	1.025	**33.401**	7792	0.458
41				**35.508**	68147	1.154	**35.509**	3599	0.212
42				**36.653**	44582	1.007	36.634	1121	0.066
43	**2.143**	104096	1.314						
44	**3.39**	295500	3.731						
45	**5.048**	186260	2.352						
46	**8.079**	483413	6.103						
47	**10.203**	99180	1.252						
48	**11.595**	228447	2.884						
49	**11.595**	228447	2.884						
50	**12.684**	475528	6.004						
51	**12.684**	475528	6.004						
52	**27.21**	82113	1.037						
53	**29.425**	111704	1.41						
54	**31.602**	142055	1.793						
55	**32.154**	111333	1.406						
56	**35.596**	112312	1.418						
57				**1.592**	47420	1.071			
58				**4.331**	57492	1.299			
59				**5.606**	49770	1.124			
60				**6.083**	81448	1.840			
61				**9.313**	62432	1.410			
62				**25.436**	100729	2.276			
63							**3.509**	20488	1.204
64							**7.351**	31647	1.861
65							**10.652**	29818	1.753
66							**19.067**	164644	9.680

Table 7. HPLC analysis summaries of solutions lixiviated from LcWT07-2 coated mature seeds, dehusked mature seeds, and seed coats.

Saturation sample	**SS-SC**	**SS-S**	**SS-C**
Total peak No.	109	51	53
Total area	8950211	1291966	1064637
Showing peak No.	16	15	14
Showing peak percentage (%)	14.68	29.41	26.42
Shown peak area	6144121	1091354	905872
Shown peak area percentage (%)	68.65	84.47	85.09

of saturation treatment. However, due to the concentration difference of the saturated coated seeds compared to saturated seed coats and dehusked seeds as earlier described, the pH values of solutions lixiviated

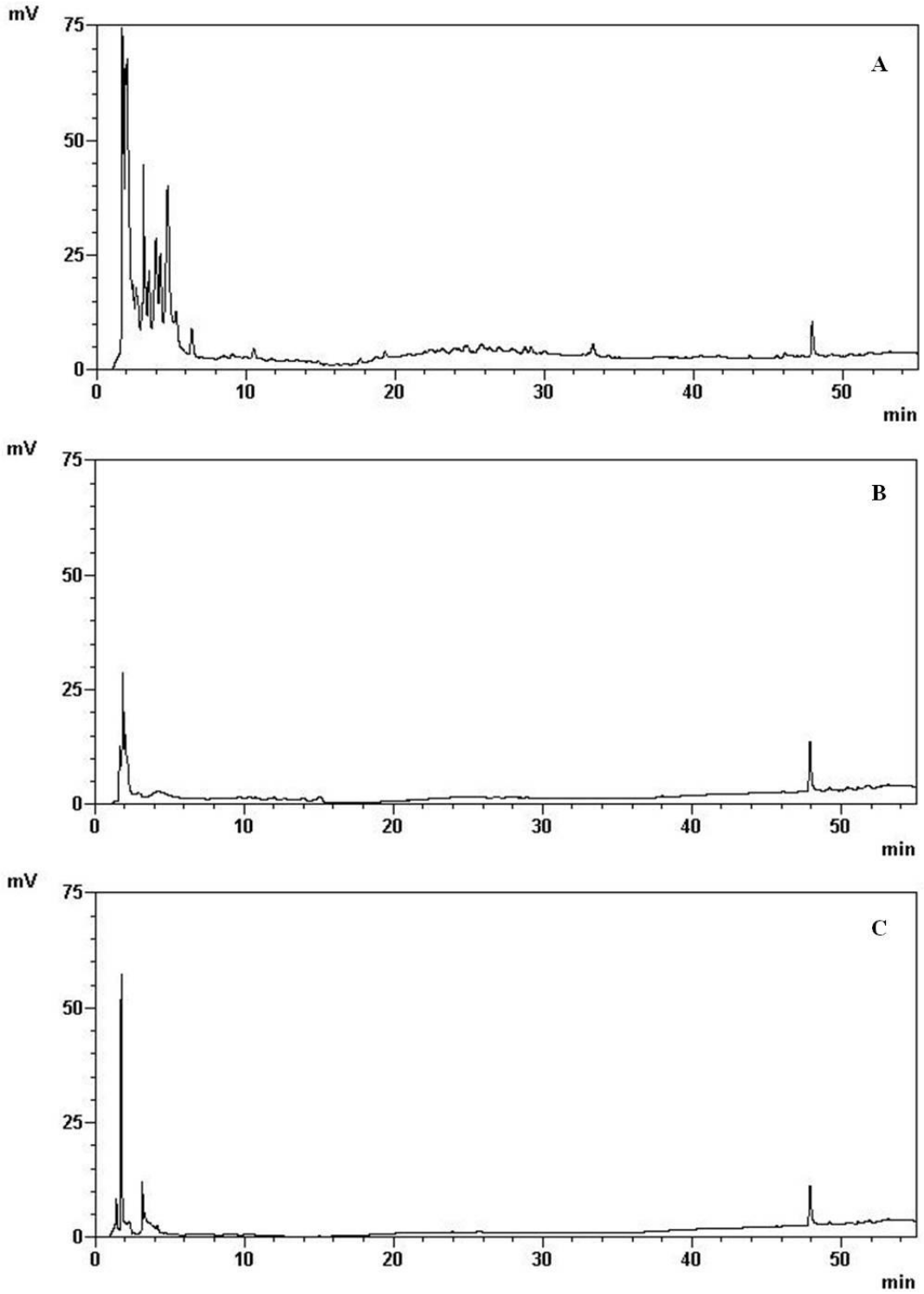

Figure 2. HPLC analysis of solutions lixiviated from LcWT07-2 coated mature seeds (A), dehusked mature seeds (B) and seed coats (C).

from seed coats were a bit higher than those of seed coats and dehusked seeds. Moreover, as the saturation length increased, the pH values of lixiviated solutions decreased regardless of the saturation sample.

Conclusion

In this study, the capacity of saturation treatments in 4°C sterile water to break seed dormancy and increase

Table 8. HPLC analysis of main peaks in solutions lixiviated from LcWT07-2 coated mature seeds, dehusked mature seeds, and seed coats.

Peak number	SS-SC			SS-S			SS-C		
	Ret. Time	Area	Area%	Ret. Time	Area	Area%	Ret. Time	Area	Area%
1	**1.728**	811515	9.067	**1.685**	110022	8.516	**1.754**	349983	32.873
2	**3.157**	524573	5.861	**3.101**	26985	2.089	**3.174**	85861	8.065
3	**3.526**	266431	2.977	**3.523**	15610	1.208	**3.592**	16075	1.51
4	**47.941**	94539	1.056	**47.91**	125379	9.704	**47.91**	102753	9.651
5	**50.482**	6432	0.072	**50.448**	14675	1.136	**50.453**	5710	0.536
6	**51.783**	11720	0.131	**51.75**	15469	1.197	**51.759**	12201	1.146
7	**53.177**	10205	0.114	**53.148**	13768	1.066	**53.151**	11678	1.097
8	**54.013**	14203	0.159	**53.971**	17377	1.345	**53.991**	7101	0.667
9	**1.936**	343230	3.835	**1.892**	202993	15.712			
10	**2.047**	1009314	11.277	**2.065**	229565	17.769			
11	**4.286**	331543	3.704	**4.238**	197278	15.27			
12	**25.818**	156153	1.745	**25.805**	5138	0.398			
13	**3.99**	491792	5.495				**3.928**	22198	2.085
14	**4.774**	861166	9.622				**4.71**	14746	1.385
15	**5.335**	362579	4.051				**5.358**	3141	0.295
16				**1.424**	12702	0.983	**1.433**	91993	8.641
17				**2.596**	23000	1.78	**2.532**	15746	1.479
18	**2.431**	175417	1.96						
19	**2.666**	325454	3.636						
20	**6.388**	180165	2.013						
21	**23.151**	106097	1.185						
22	**26.959**	104153	1.164						
23				**2.959**	40036	3.099			
24				**6.013**	26722	2.068			
25				**15.094**	32475	2.514			
26							**2.286**	51550	4.842
27							**3.342**	57708	5.42
28							**3.665**	37164	3.491
29							**4.158**	36216	3.402

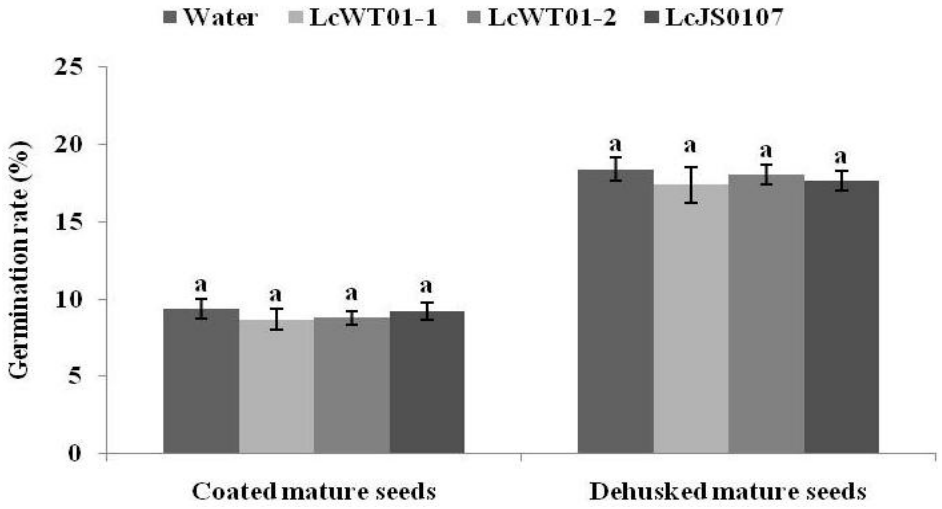

Figure 3. The effect of water and solutions lixiviated from coated mature seeds on germination rates of LcJS0107 coated and dehusked mature seeds.

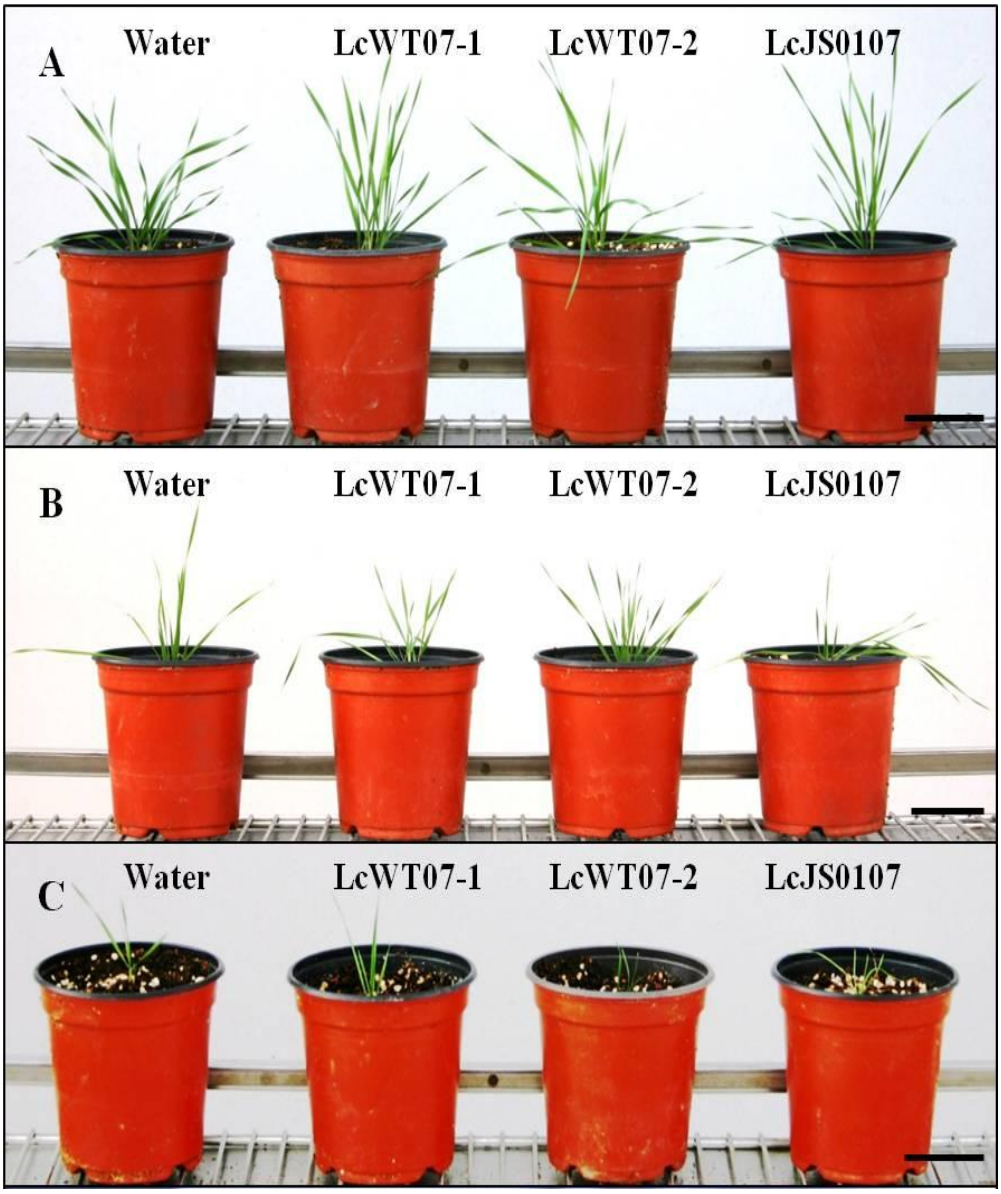

Figure 4. The effect of plant growth and development after treatment with water or lixiviated solutions from LcWT07-1, LcWT07-2 or LcJS0107 coated mature seeds. A, B and C: LcJS0107 plants after 1-week of watering treatment; D, E and F: LcJS0107 plants after 4-week of watering treatment. Scale bar: 10 cm.

germination rate in *L. chinensis* was investigated. Saturation treatment was predicted to lixiviate germination inhibitors from seed coats and/or mature seeds to break seed dormancy and increase germination rates.

As predicted, the lixiviated solutions had inhibiting effects on *L. chinensis* seed germination rates. UV and HPLC analysis indicated that the lixiviated solutions comprised of discrete compounds, some of which were unique to particular seed components.

Moreover, since *L. chinensis* is a halophyte capable of germinating and growing in highly alkali-sodic soils naturally, we hypothesize that the acidic components lixiviated from seed coats and/or mature seeds contribute to the neutralization of environmental salinity and alkalinity to retain normal growth in environmentally stressed conditions.

Validation of this hypothesis will require further studies on the characteristics and function of *L. chinensis* lixiviated components.

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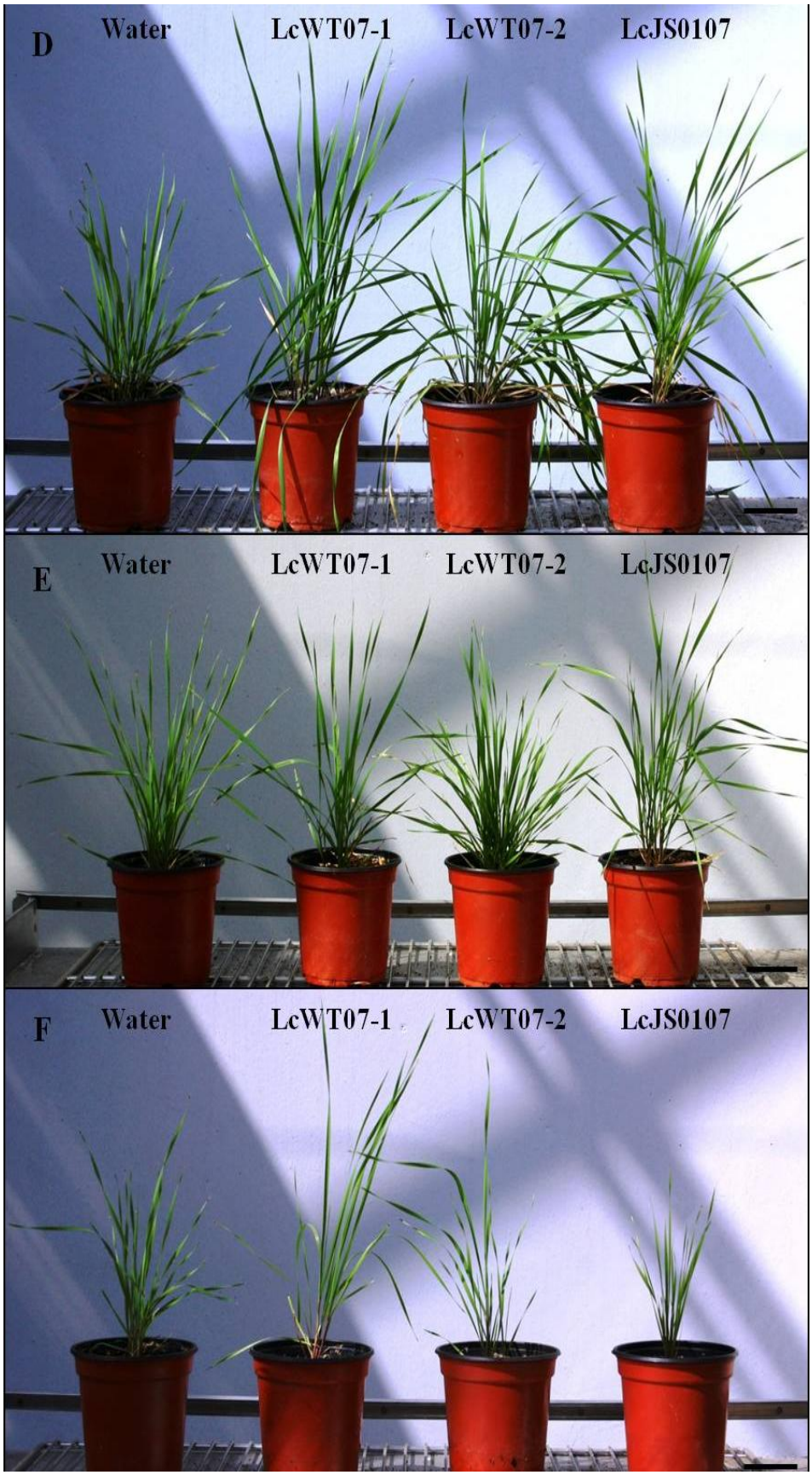

Figure 4. Contd.

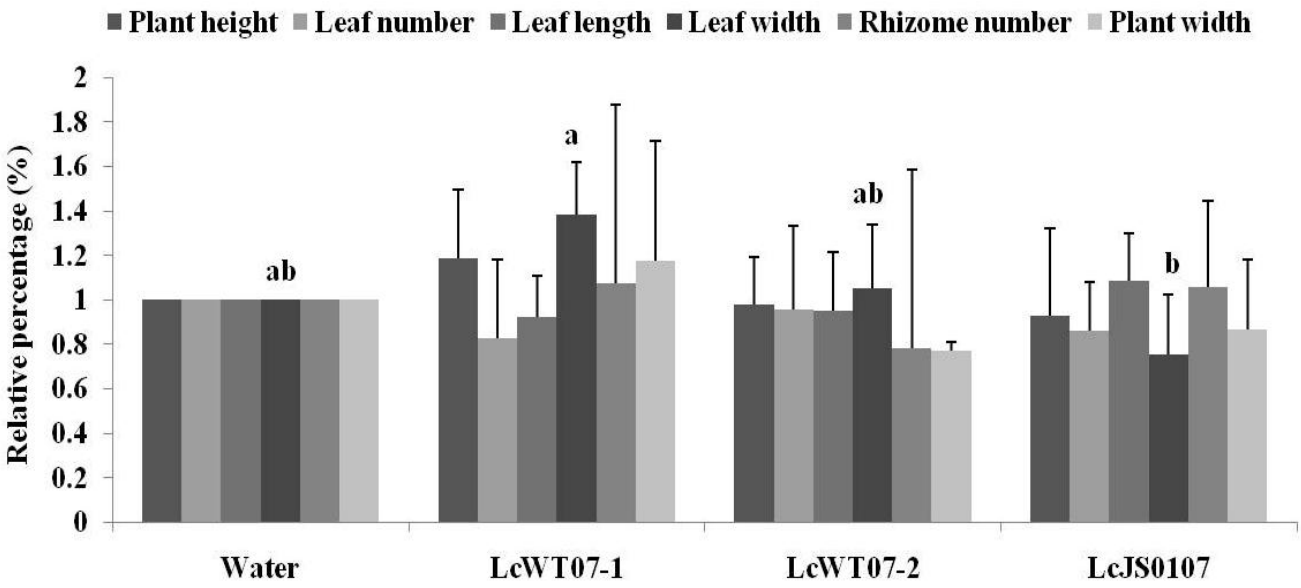

Figure 5. The effect of plant height, leaf number, leaf length, leaf width, rhizome number and plant width after treatment with water or lixiviated solutions from LcWT07-1, LcWT07-2 or LcJS0107 coated mature seeds, presented as a relative percentage using the effects due to water treatment as controls. Means followed by the same lowercase letter in the same column are not significantly different at $P < 0.05$ according to two-way ANOVA using Duncan's multiple-range test.

Figure S1. Morphology of LcWT07-1, LcWT07-2 and LcJS0107 coated mature seeds.

Table 9. The pH values of lixiviated solutions.

Lixiviated solutions	Plant genotype	Saturation period (day)		
		1	2	3
Water			6.56	
SS-SC	LcWT07-1	5.87	5.26	4.67
	LcWT07-2	6.01	5.34	4.98
	LcJS0107	6.11	5.28	4.82
SS-S	LcWT07-1	5.12	5.16	4.79
	LcWT07-2	5.12	5.12	3.92
	LcJS0107	4.96	4.78	4.16
SS-C	LcWT07-1	5.45	5.11	4.67
	LcWT07-2	6.5	5.51	5.19
	LcJS0107	5.67	5.45	5.21

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