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Effect of seed invigoration by osmo-conditioning on the radicle emergence and the physiological parameters of the true seed of shallots (*Allium ascalonicum* L.)

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Abstract: Seed deterioration resulting from production and storage factors is the major cause of differences in the seed vigour that results in low seedling emergence in both the laboratory and in the field. An experiment was conducted to evaluate the effect of seed invigoration by osmo-conditioning on the radicle emergence and the physiological parameters of naturally deteriorated seed lots of the true seed of shallots. The experiments were arranged in a completely randomised design with a repeated measurement for radicle emergence and a randomised complete block design for seedling vigour in the field with four replicates. The results indicated that the radicle emergence of the invigorated seed with $ZnSO_4$ correlated well with the seed viability and vigour parameters for seed lots stored under uncontrolled conditions. The radicle emergence of invigorated seeds stored under the uncontrolled condition were more predictive and strongly correlated with the seedling vigour parameters in the field, i.e., the field emergence, field emergence rate and mean emergence time with $r = 0.968$, $r = 0.970$ and $r = –0.947$, respectively. Furthermore, the coefficients of determination were significant ($P < 0.05$) with $R^2 = 0.936$ field emergence, $R^2 = 0.941$ field emergence rate and $R^2 = 0.898$ for the mean emergence time. Seed invigoration with 0.5% $ZnSO_4$ further significantly reduced the time of a single count of RE from 72 hours and 68 hours to 60 hours with field prediction rates ranging from 90–99%. It was concluded that the radicle emergence of TSS can be improved by invigorating the seeds with 0.5% $ZnSO_4$ thereby increasing the germination percentage, vigour index, and germination rate and reducing the mean germination time.

Keywords: field emergence; germination rate; mean germination time; viability; vigour index

The shallot *(Allium ascalonicum* L.) is an important vegetable crop produced mainly for home consumption and income generation by smallholder farmers in tropical and subtropical countries. However, the scarcity of high-quality seed and yielding varieties seriously constrains the productivity of the crop, i.e., there is no guaranteed availability of the true seed of shallots (TSS), i.e., the planting material with high quality that can produce high-yield up to 19 tonnes/ha (Shimeles 2014; Idhan et al. 2015). Furthermore, the use of TSS in most countries is limited by the availability of quality and vigorous seeds at the time of sowing which is attributed to the low production of quality seeds, and poor storage under fluctuating ambient temperatures and relative humidity. This poses a chal-

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lenge for storing shallot seeds for longer periods which results in their deterioration and eventually in a loss of viability and vigour.

The germination of onion seeds, for example, especially under stressful conditions, such as low temperatures, continues to be a matter of significant importance to growers who plant the seed directly in the field. Seed enhancement through invigoration or priming has resulted in a significant improvement in the farmers' ability to achieve this goal both in an open and controlled environment (Amin et al. 2016). Seed invigoration is a pre-sowing, controlled-hydration environment in which seeds are exposed to an external water potential that is sufficiently low enough to prevent radicle protrusion, but is able to stimulate physiological and biochemical activities (Adetunji et al. 2021). Rapid and uniform emergence and germination are vital factors that increase the yield, seed quality and profits for farmers. Khan et al. (2016) observed that crop uniformity and the percentage of normally germinated seedlings have a major effect on the final yield and quality. Seeds that delay or take a long time to germinate tend to expose the planting media to deterioration and soil compaction under unfavourable environmental conditions. However, the seed vigour can be improved through seed invigoration, i.e., by soaking the seeds in KNO_3, PEG 6000 (Castañares, Bouzo 2018), GA_3, $ZnSO_4$ and other osmotic solutions prior to planting (Kharat et al. 2022). Research has shown that seed invigoration increases germination and the seedling establishment of onion seeds as compared to those of seed bulb crops, thereby ensuring uniformity and a specific growth rate (Mirabi, Hasanabadi 2012; Agung, Diara 2017). Seed invigoration has been proved to speed up germination and can be used to speed up the radicle emergence (RE) and reduce the mean germination time (Castañares, Bouzo 2018). According to Owen and Pill (1994), primed asparagus and tomato seeds maintained maximum germination, germination rate, and high vigour after three months of storage when the temperature was held at 4 °C rather than at 20 °C. However, Basra et al. (2005) noted that primed canola seeds maintained their increased vigour by six months storing at low temperature conditions (8 °C).

The role of KNO_3 and $ZnSO_4$ in promoting germination has been reported in tomato seeds (Lara et al. 2014) and barley (Sharkawy et al. 2017). According to Sevarani and Umarani (2011) and Jagosz (2015), the use of osmo-conditioning and pre-sowing treatment methods has been successful in improving the speed of germination, increasing the germination percentage, and reducing the number of abnormal seedlings of onions (*Allium cepa* cv. Aggregatum L.) in the field. Seed invigoration treatments have also been reported to increase the germination capacity of parsley (*Petroselinum crispum* L.) (Dursun, Ekinel 2010), tomatoes (*Lycopersicum esculentum* L.) (Mirabi, Hasanabadi 2012); and carrots (*Daucus carota* L.) (Sevarani, Umarani 2011). Hard seed coats and high temperatures during sowing may affect or inhibit germination in most crop seeds. However, seed invigoration techniques may be used as an important method to enhance seed germination, performance and be able to withstand harsh field environments especially when the environment is not conducive for germination (Shal 2007). Therefore, this study was conducted to evaluate the effect of seed invigoration by osmo-conditioning on the RE and other physiological parameters of naturally deteriorated seed lots of the TSS.

METHOD

To study the effect of seed invigoration with zinc sulfate on the radicle emergence and other physiological parameters of the TSS, an experiment was conducted in the laboratory and at the student's research farm of the Department of Agronomy and Horticulture, IPB University in 2021. The experiment used the TSS variety Trisula which was produced at an experimental station of the Indonesian Vegetables Research Institute (IVEGRI) in North Sumatra and was harvested on the 3rd of October 2020 with an initial germination of 94.6%. The experiment further used the seeds which were packaged in six different materials, namely, aluminium foil, a glass bottle (GB), a khaki envelope, plastic paper, a plastic bottle, and a polyethylene sack and stored under two different storage conditions, i.e., controlled condition (CC) and uncontrolled condition (UC) with the temperature and relative humidity (RH) ranging from 17.5 °C–19.5 °C and RH from 55%–65.5% for the CCs, and 26–32 °C , RH 61–79% for the UCs. After 6 months, the seeds were invigorated with 0.5% $ZnSO_4$ before being subjected to RE and physiological testing.

The experiments were arranged in a completely randomised design (CRD) for the laboratory vigour tests with two factors, i.e., packaging materials having six levels and storage conditions having two levels which

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were replicated four times. For the radicle emergence (RE), experiments were arranged in a CRD with repeated measurements/observations, RE observations were taken every 4 hours at least 9 times at different emergence times starting from 48 hours after sowing up to 80 hours (from the pre-experiment). For the radicle emergence and physiological parameters, 100 seeds were placed on filter paper in containers and kept in a standard germinator at 25 ± 1 °C. The seedling vigour tests in the field, such as the field emergence rate (FER), mean emergence time (MET), and viability test, i.e., field emergence (FE), were conducted and arranged in a randomised complete block design (RCBD) with four replicates using 100 seeds per replicate.

Seed osmo-conditioning was conducted using 0.5% $ZnSO_4$ for 10 hours according to Aboutalebian et al. (2012). Seed invigorations were performed in plastic bottles (200 ml) where the seeds were placed. Small holes were created at the top of each bottle to allow aeration and the shaking of the bottles was conducted every 30 minutes during the period of invigoration. After the invigoration treatment, the seeds were surface washed three times with distilled water and dried closer to the original moisture with forced air under uncontrolled conditions and then sowed. Observations were made on the viability and vigour, i.e., data were recorded on the radicle emergence (RE), germination percentage of normal seedlings (GP), mean germination time (MGT), vigour index (VI) and germination rate (GR). The following treatments were established (Table 1).

The radicle emergence (%) was calculated by dividing the number of newly emerged seeds at a given time by the total number of seeds sown multiplied by 100. The germination percentage (%) was calculated according to the International Seed Testing Association (ISTA) (2014) by summing up the normal seedlings on the first and final counts (6 and 12 days after sowing, respectively), then dividing by the total number of seeds planted multiplied by 100.

The mean germination time (MGT) was calculated by the formula given by Ellis and Roberts (1981).

$$\text{MGT} = \frac{\Sigma\,(n \times D)}{\Sigma\,(n)} \quad (1)$$

where: n — number of newly emerged seeds (radicle protrusion = 2 mm) at time D; D — days from the beginning of the emergence test;
Σn — final number of germinated seeds.

The vigour index (VI) tests were determined according to ISTA (2014), i.e., the number of normal seedlings germinated on the first count (6 days after sowing) expressed as a percentage. The speed of germination (germination rate) was calculated according to Maguire (1962) and Sadeghianfar et al. (2019).

$$\text{Germination rate} = \frac{X_1}{Y_1} + \frac{X_2}{Y_2} + \ldots + \frac{X_n}{Y_n} \quad (2)$$

where : X_1, X_2 and X_n — number of seeds with radicle protrusion on the 1st, 2nd, and n^{th} hour, respectively;
Y_1, Y_2, and Y_n — number of hours from sowing to the 1st, 2nd, and n^{th} count, respectively.

The field emergence, field emergence rate and mean emergence time for the seedling vigour tests in the field were observed and recorded.

An analysis of variance was performed to determine the level of significance, and Duncan's Multiple Range Test (DMRT) at a 5% level of significance was used to compare the mean value between the treatments on the observed indicator of the RE. A simple linear regression analysis was performed to obtain the correlation coefficients and coefficient of determination on the treatment means in order to determine the relationship between the radicle emergence, germination percentage, seedling field emergence and the other vigour tests. The R-Statistical (2021) package and Microsoft excel programs were used in the data analysis.

Table 1. Seed lot classifications according to the type of storage material and conditions

Controlled condition (CC)	Uncontrolled condition (UC)
AL-$_{cc}$ = Aluminium foil + CC	AL-$_{uc}$ = Aluminium foil + UC
GB-$_{cc}$ = Glass bottle + CC	GB-$_{uc}$ = Glass bottle + UC
EV-$_{cc}$ = Khaki envelopes + CC	EV-$_{uc}$ = Khaki envelopes + UC
PP-$_{cc}$ = Plastic paper + CC	PB-$_{uc}$ = Plastic bags + UC
PB-$_{cc}$ = Plastic bottles + CC	PB-$_{uc}$ = Plastic containers + UC
SK-$_{cc}$ = Polyethylene sack + CC	SK-$_{uc}$ = Polyethylene sacks + UC

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RESULTS AND DISCUSSION

Effect of seed invigoration with $ZnSO_4$ on the vigour and viability of the TSS. The results indicated that the seed invigoration with $ZnSO_4$ increased the germination percentage from 45.5% to 81.5% for seeds that were stored in aluminium (AL) foil under controlled conditions, and by 26.0% for seeds stored in the same material in uncontrolled conditions, i.e., from 53.0% to 79.0% (Table 2). Furthermore, significant differences were observed in the packaging material between the invigorated and non-invigorated seeds, with the seeds packaged in a plastic bottle having low germination (70.0%) and (52.0%) for the invigorated and non-invigorated seeds, respectively. However, the seeds that were stored under the uncontrolled conditions were not significant among the packaging materials except AL. According to ISTA (2014), seeds that have already deteriorated cannot be improved by priming or invigoration which is in line with seed lots stored under uncontrolled conditions.

The results further indicated that the MGT was reduced in both packaging materials with seed lots stored under the controlled conditions germinated earlier as compared to seeds stored under the uncontrolled conditions (Table 2). Seeds packaged in AL had 2.17 days (130 hours) and 2.21 days (132 hours) for seeds stored at the controlled and uncontrolled conditions, respectively. The speed of germination (germination rate) was significant among the treatments with the seeds stored under the controlled and uncontrolled conditions packaged in AL having a higher number seeds germinated per day as compared to the rest of the treatments. The seeds that were stored under the uncontrolled conditions had a fewer number of seeds germinated per day for both the invigorated and non-invigorated seeds ranging from 3.1 to 6.9 except for the seeds packaged in AL with a minimum of 16.4 and 18.9, which was significantly higher for the non-invigorated and invigorated seeds, respectively.

The improved germination rate for the invigorated seeds in this research was due to the metabolic repair during imbibition which has been described as a build-up of germination-enhancing metabolites (Basra et al. 2005). Pre-germination activities inside the seed are considered as the main factors that determine the rate at which the seed will germinate. During imbibition, enzymes must be activated to initiate the process of germination. When seeds are soaked in water, an osmotic adjustment is considered as one of the main merits of the soaking treatment to increase the germination and emergence rate. Research has shown that pre-sowing with hydration treatments improved the seed germination by delivering various enzymatic activities to produce energy for respiration. Seed invigoration with $ZnSO_4$ can improve the seed emergence, stand establishment and subsequent growth and yield. According to Babaeva et al. (1999), the primed seed of *Echinacea purpurea* L. with a 0.5% $ZnSO_4$ solution increased the germination and field emergence by 38 and 41%, respectively, the results, which are similar to those in the current research, where the invigorated TSS with 0.5% $ZnSO_4$ increased the germination percentage, germination rate, vigour index and reduced the mean germination time. The reduced MGT would be subjected to the early germination of the invigorated seed due to the rapid completion of the pre-germinative, metabolic and enzymatic activities for the energy production, making the seed ready for radicle emergence and seed germination (Thejeshwini et al. 2018).The effect of seed invigoration with 0.5% $ZnSO_4$ for 10 hours before sowing followed by the partial drying significantly increased the RE and reduced the MGT in the TSS. The controlled storage conditions kept the TSS seed lots better, the seeds had higher viability and vigour six months after storage, as indicated by the higher VI, GP, GR, and smaller MGT than the seed lots stored under the uncontrolled conditions. The invigoration of seeds with $ZnSO_4$ increased the viability and vigour of the seed lots that had been stored for 6 months, regardless of the storage conditions. However, the lower the viability and vigour of the stored seeds, the lower the possibility of meeting the minimum standard quality of certified seeds. Therefore, storing TSS under controlled low temperatures would be beneficial because the seed lots would not deteriorate so that the invigoration would increase the viability to at least the minimum standard of certified seed. As such, AL foil would be the packaging material recommended for seed lots stored under uncontrolled conditions, while a GB would be the best alternative under controlled storage conditions.

Effect of seed invigoration with $ZnSO_4$ on the radicle emergence of the true seed of shallot. Significant differences were observed among the packaging materials 80 hours after sowing with AL having the highest number of radicle emergence at 81.5% (Figure 1A) than the rest of the treatments ($P < 0.05$) for the seed lots stored under uncontrolled

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Table 2. The viability and vigour of TSS 6 months after storage following seed invigoration with $ZnSO_4$

PM/SC	Inv-AL	Non-AL	Inv-GB	Non-GB	Inv-EV	Non-EV	Inv-SK	Non-SK	Inv-PP	Non-PP	Inv-PB	Non-PB	*P*-value
Vigour index (%)													
Controlled	54.5^{Ab}	35.5^{Ae}	67.5^{Aa}	43.0^{Ac}	70.0^{Aa}	17.5^{Af}	52.0^{Abc}	26.0^{Aef}	53.5^{Abc}	15.5^{Af}	50.0^{Abc}	25.5^{Aef}	*
Uncontrolled	29.0^{Ba}	29.0^{Ba}	12.0^{Bbcd}	5.0^{Bde}	12.5^{Bbcd}	1.5^{Be}	8.5^{Bcde}	4.0^{Bde}	16.0^{Bbc}	4.0^{Bde}	19.5^{Bab}	2.5^{Be}	*
P-value	*	*	*	*	*	*	*	*	*	*	*	*	
SC × PM	*	*	*	*	*	*	*	*	*	*	*	*	
Germination (%)													
Controlled	81.5Aa	44.5^{Bd}	79.5^{Aab}	56.5^{Ac}	82.5^{Aa}	42.0^{Ad}	70.0^{Ab}	50.5^{Acd}	77.5^{Aab}	52.5Acd	70.0^{Ab}	52.0^{Acd}	*
Uncontrolled	79.0Aa	53.0^{Acd}	69.0^{Bab}	23.0^{Bef}	45.0^{Bd}	45.0^{Ad}	43.0^{Bd}	12.5^{Bf}	54.0^{Bcd}	18.0Bef	64.0^{Bbc}	27.5^{Be}	*
P-value	ns	*	*	*	*	ns	*	*	*	*	ns	*	
SC × PM	*	ns	ns	*	*	ns	*	*	*	*	ns	*	
Mean germination time (days)													
Controlled	2.17Af	2.44^{Ac}	2.23^{Bef}	2.46^{Bc}	2.73^{Ba}	2.72^{Ba}	2.29^{Bdef}	2.65^{Bab}	2.31^{Bd}	2.59^{Bb}	2.30^{Bde}	2.67^{Ba}	*
Uncontrolled	2.21Af	2.55^{Ae}	2.59^{Aed}	2.94^{Aab}	2.93^{Acd}	3.06^{Aa}	2.77^{Abcd}	3.04^{Aa}	2.61^{Ade}	3.05^{Aa}	2.76^{Abcd}	2.89^{Aabc}	*
P-value	ns	ns	*	*	*	*	*	*	*	*	*	*	
SC × PM	*	*	*	*	*	*	*	*	*	*	*	*	
Germination rate (seed/day)													
Controlled	17.4^{Ab}	$15.2A^{cd}$	18.8^{Aa}	15.9^{Abc}	17.8^{Aab}	13.6^{Ade}	16.9^{Abc}	11.9^{Ae}	18.8^{Aa}	15.3^{Acd}	16.2^{Abc}	15.2^{Acd}	*
Uncontrolled	18.9^{Aa}	16.4^{Ab}	6.9^{Bbc}	7.9^{Bc}	4.4^{Be}	4.3^{Be}	3.7^{Be}	3.7^{Be}	5.1^{Bde}	3.5^{Be}	4.8^{Bde}	3.1^{Be}	*
P-value	ns	ns	*	*	*	*	*	*	*	*	*	*	
SC × PM	*	ns	*	*	*	*	*	*	*	*	*	*	

The numbers preceding the same small letters for the packaging material (rows) and the numbers preceding the same capital letters for the storage condition (column) are not significantly different

*level of significance; $P < 0.05$; ns – not significant; Inv – invigorated seed lots; non – non-invigorated seed lots; PM/SC – packaging material/storage condition; AL – aluminium foil; GB – glass bottle; EV – khaki envelopes; SK – polyethylene sack; PP – plastic paper; PB – plastic bottles

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condition. Furthermore, seeds packaged in polyethylene (SK) had the lowest RE which was not significant as compared to the plastic bottle (PB), plastic paper (PP), khaki envelope (EV), and GB with an RE of 26.0%, 26.0%, 23.5% and 34.5%, respectively. The results further indicated that the seed lots stored under controlled conditions had the highest RE as compared to the seed lots stored under uncontrolled conditions (Figure 1B), However, the GB recorded the highest RE% than the rest of the treatments. The seed invigoration with $ZnSO_4$ did not improve the RE for seeds packaged in the GB, EV, SK, PP, and PB stored under uncontrolled conditions (Figure 1A). This is an indication that the seed had already deteriorated. According to Kapoor et al. (2011), seed deterioration is inexorable, continuous and irreversible. As such, it is impossible to recover the physiological seed level lost during handling and storage of seeds after harvesting by any seed treatment.

Correlation and regression analysis between the RE and seed vigour parameters of the invigorated TSS with $ZnSO_4$. Strong and significant correlation coefficients were observed with the seeds stored under controlled conditions having higher correlation values for the VI (0.822), GP (0.824), MGT (–0.923) and GR (0.995) 60 hours after sowing as compared to the seed lots stored under uncontrolled conditions with 0.628, 0.679 and –0.902 for the VI, GP and MGT, respectively (Table 3). However, the correlation values for the mean germination time (MGT) and germination rate (GR) were statistically similar at both storage conditions with correlation coefficients ranging from –0.902 to –0.923 for the MGT and 0.995 to 0.998 for the GR. The correlation coefficients at 60 hours after sowing were significantly higher at both storage conditions and were selected for a single count of radicle emergence to predict the seed vigour parameters in the laboratory and the seedling vigour in the field. The effect of the seed invigoration with 0.5% $ZnSO_4$ significantly reduced the time at which a single count of RE can be observed by 16.7% from 72 hours to 60 hours (Kamanga et al. 2021). The correlations between the RE and the other vigour parameters, such as MGT and VI, were logical as they all reflect the rate of the germination in the field which was an indication that the RE can be used as a method for the seed vigour test. Previous research on radicle emergence indicated that the RE of sweet maize was highly related to the mean germination time (MGT), germination emergence (GE), germination

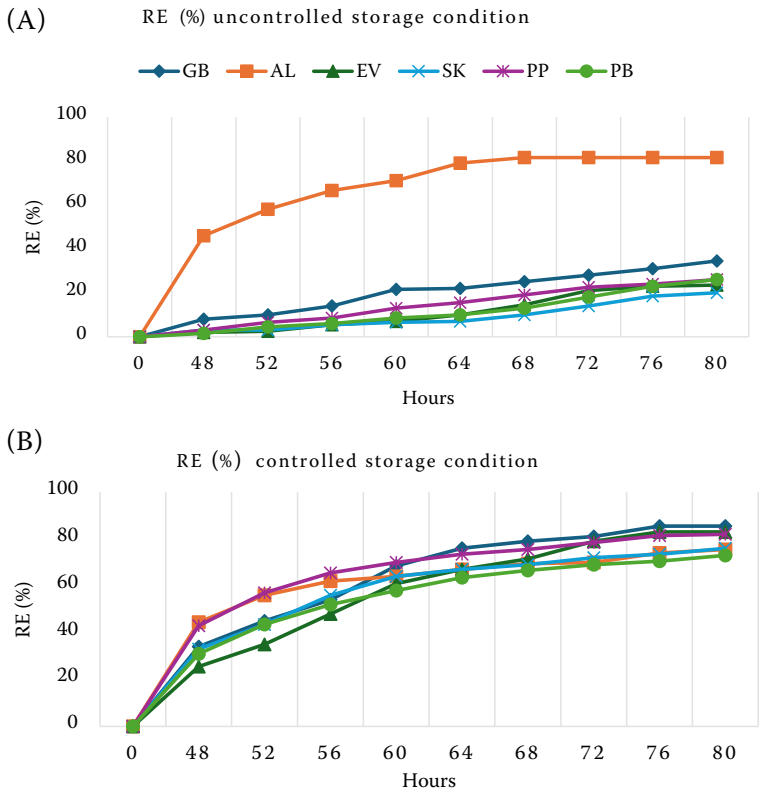

Figure 1. Effect of the seed invigoration with $ZnSO_4$ on the radicle emergence of TSS 6 months after storage
RE – radicle emergence

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Table 3. Correlation analysis of the RE of the invigorated TSS and seed vigour parameters

Parameter	Period of observation (h)							
	48	52	56	60	64	68	72	76
Seed storage under uncontrolled conditions								
VI	0.561**	0.588**	0.594**	0.628**	0.616**	0.625**	0.655**	0.671**
GP	0.633**	0.679**	0.655**	0.679**	0.667**	0.662**	0.663**	0.687**
MGT	–0.850*	–0.882*	–0.891*	–0.902*	–0.892*	–0.893*	–0.851*	–0.833*
GR	0.974**	0.973**	0.986**	0.998**	0.989**	0.991**	0.991**	0.993**
Seed storage under controlled conditions								
VI	0.722**	0.752**	0.793**	0.822**	0.822**	0.822**	0.855**	0.867**
GP	0.771**	0.787**	0.797**	0.824**	0.828**	0.829**	0.827**	0.835**
MGT	–0.916*	–0.923*	–0.934*	–0.923*	–0.917*	–0.915*	–0.885*	–0.865*
GR	0.934**	0.952**	0.977**	0.995**	0.993**	0.991**	0.993**	0.992**

**level of significance $P < 0.01$; * – $P < 0.05$; VI – vigour index; GP – germination percentage; MGT – mean germination time; GR – germination rate

percentage (GP), germination index and vigour index, per Zhao et al. (2007).

The regression analysis indicated that a single count of radicle emergence 60 hours after sowing was predictive of the germination percentage having a coefficient of determination of $R^2 = 0.895$ for the seeds stored under uncontrolled conditions. However, the invigorated seed lots stored under controlled conditions were unable to predict the germination of normal seedlings with $R^2 = 0.132$ (Figure 2A). These results showed that seed invigoration with 0.5% $ZnSO_4$ improved the radicle emergence and the germination rate, and reduced the mean germination time of the true seeds of shallot.

The results further indicated that a single count of RE at 60 hours was predictive of the vigour index for the seeds stored under the uncontrolled condition with a coefficient of determinations $R^2 = 0.702$ and failed to predict those stored under the controlled condition with $R^2 = 0.06$ which was insignificant (Figure 2B). The coefficient of determinations for the mean germination time were all predictive with $R^2 = 0.969$ and $R^2 = 0.642$ for the seeds stored under uncontrolled and controlled conditions, respectively (Figure 2C). A single count of RE of invigorated seeds at 60 hours was able to predict the germination rate with $R^2 = 0.996$ for the seeds stored under uncontrolled conditions and $R^2 = 0.770$ for the seed lots stored under controlled conditions (Figure 2D). The results revealed that invigorated seeds regained seed vigour and sped up the germination process of the TSS.

Previous research has shown that seeds with high vigour normally produce good and uniform seedlings with high germination levels and hence cannot be used to predict other seed vigour parameters. Lv et al. (2016) reported that seed lots with high vigour of 15 commercial seed lots of both *A. sativa* and *E. nutas* were unable to predict the germination percentage and field emergence. According to Matthews and Khajeh-Hosseini (2007), the differences in the RE rates which determine the MGT were caused by the length of the delay from the start of the imbibition to the RE. This delay has been interpreted as dependent upon the time required for metabolic repair before radicle emergence. The greater delay before the RE in an aged seed suggests that they require more time to repair for previously sustained deterioration, and hence require more time from the start of imbibition to RE (Mathews et al. 2012).

The radicle emergence of invigorated seeds stored under uncontrolled conditions were more predictive and strongly correlated with the seedling vigour parameters in the field, i.e., the field emergence (FE) (Figure 3A), field emergence rate (FER) (Figure 3B) and mean emergence time (MET) (Figure 3C) with their respective coefficient of correlation $r = 0.968$, $r = 0.970$ and $r = —0.947$. Furthermore, the coefficients of determination were significant *(*$P < 0.05$) with $R^2 = 0.936$, $R^2 = 0.941$ and $R^2 = 0.898$ for the FE, FER and MET, respectively (Figure 3). On the contrary, the RE of the seed lots stored under controlled conditions did not correlate well with the seedling vigour parameters in the field. Furthermore, the regression analysis results showed that the coefficients of determination were not predictive with $R^2 = 0.042$, $R^2 = 0.266$ and $R^2 = 0.047$ for the FE, FER and MET, respectively, which were all insignificant

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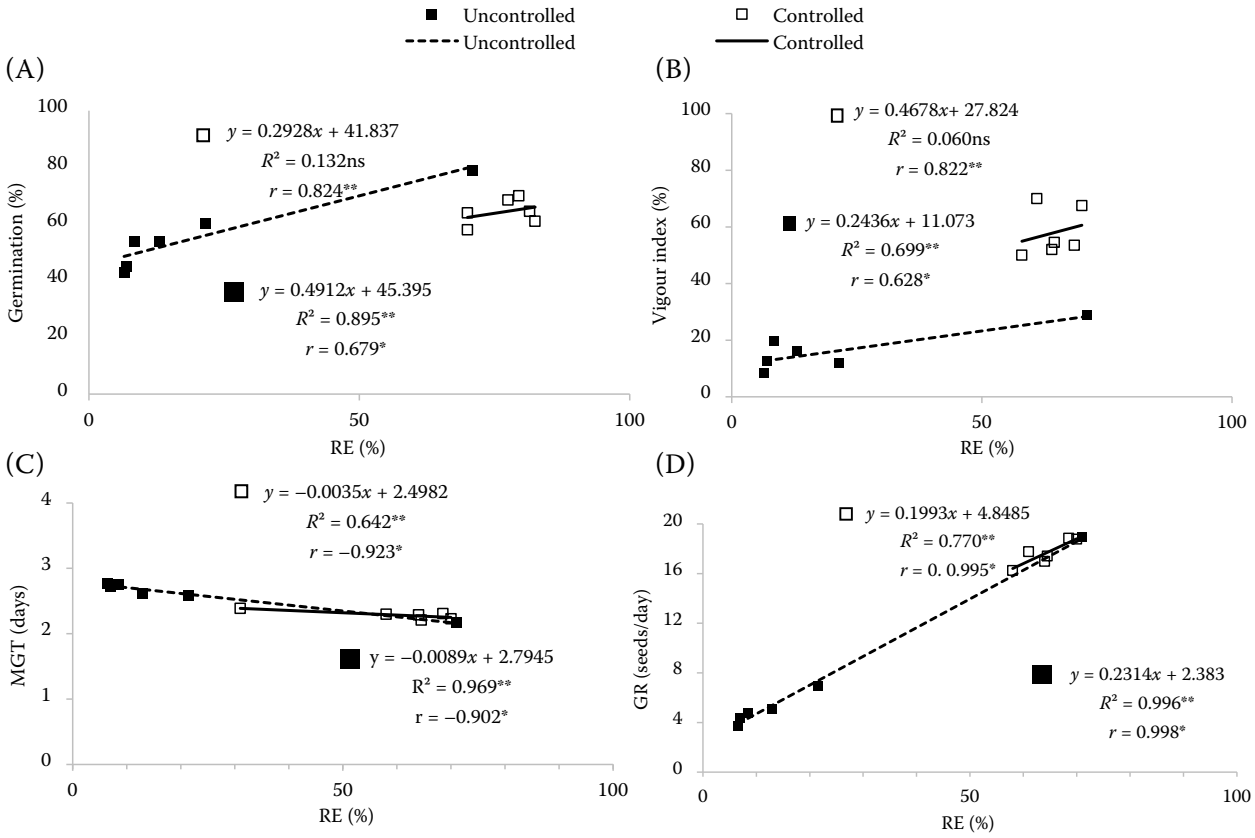

Figure 2. Correlation and regression analysis of the RE of the invigorated seed lots and seed vigour in the laboratory (A) germination, (B) vigour index;, (C) mean germination time (MGT), (D) germination rate

*level of significance; $P < 0.05$; **$P < 0.01$; ns — non significant; RE — radicle emergence; GP – germination percentage

($P > 0.05$) for seed lots stored under controlled conditions.

The current results show that a single count of RE for invigorated seeds stored under uncontrolled conditions can be used to predict the germination percentages in the laboratory and field emergence (Figure 3). Seed lots which were stored under uncontrolled conditions were able to predict the field emergence (93.6%) and mean emergence time (89.8%) and field emergence rate (94.1%) for a single count of RE at 60 hours after sowing than it was previously predicted with non-invigorated seed lots at 72 hours after sowing (Kamanga et al. 2021). These results are higher than what Ermis et al. (2015) found in leek seeds for a single count of RE at 120 hours for emergence in modules (R^2 = 0.701) and the mean emergence time (R^2 = 0.65), and still concluded that a single count of radicle emergence could be used to predict the seedling emergence potential in leek seeds. However, the findings in this research differ from previous studies in such a way that seed lots which were stored under controlled conditions maintained high vigour and did not correlate well and predict the field emergence (R^2 = 0.042), mean emergence time (R^2 = 0.047) and field emergence rate (R^2 = 0.266). The findings in this research concur with Lv et al. (2016) who reported similar findings on seed lots of both *A. sativa* and *E. nutas.*

Research has demonstrated that the time and osmotic solution's main effects were found to be important with respect to emergence. For example, all primed treatment solutions with PEG 6000 and 2% KNO_3 resulted in higher germination percentages as compared to non-primed seeds (Arin et al. 2011). This demonstrates that the seed treatment is a tool to recondition damaged tissues and prepare seeds for germination. Jagosz (2015) compared untreated seeds by using both a PEG 6000 and PEG 8000 osmotic which similarly increased the percentage of normal seedlings, reduced the percentage of abnormal seedlings and shortened the MGT, the results which are similar to the findings in this research.

Seed invigoration has been recommended as one way of improving seed vigour. The seed treatments

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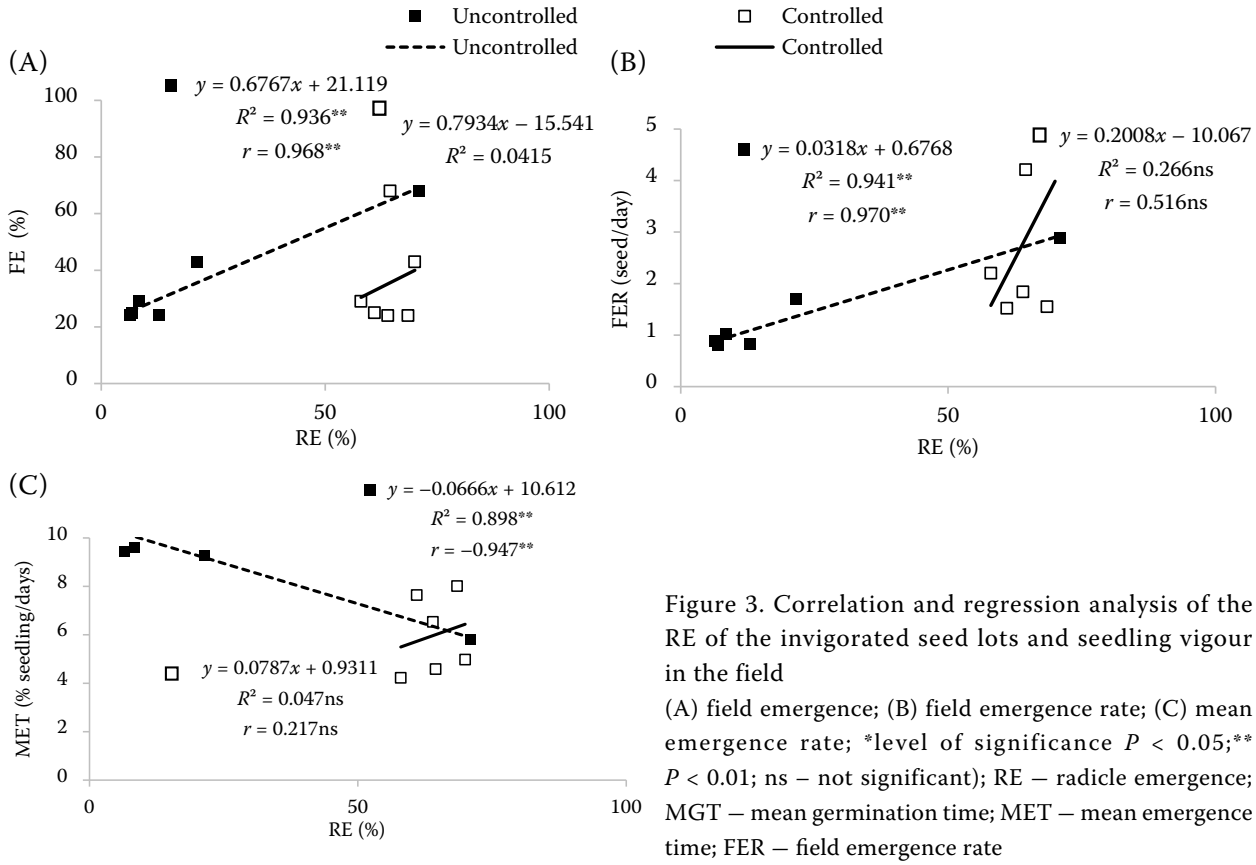

Figure 3. Correlation and regression analysis of the RE of the invigorated seed lots and seedling vigour in the field

(A) field emergence; (B) field emergence rate; (C) mean emergence rate; *level of significance $P < 0.05$;** $P < 0.01$; ns – not significant); RE — radicle emergence; MGT — mean germination time; MET — mean emergence time; FER — field emergence rate

in this research proved to be effective in improving the RE and reducing the time for a single count of RE from 72 hour as reported by Kamanga et al. (2021) to a 60-hour period. Storage food reserves, such as carbohydrates and proteins, play a key role in the seed during the process of imbibition and germination, exposing the seed to high temperatures and relative humidity can cause changes in the protein structure and cause it lose its biological functions. Seed lots stored under uncontrolled conditions were prone to physiological damage due to adverse storage conditions. Non-viable seeds were unable to synthesise enough protein during imbibition and prolonged the MGT of the seed (Bewley et al. 2013). This may be a consequence of damage to the DNA and is usually accompanied by reducing the ability to synthesise DNA and resulting in decreased seed germination (Marcos-Filho 2016).

An increased germination rate and seedling emergence in the field are beneficial to farmers, especially more so if the seeds have been stored for a relatively long period. During storage, seeds can be exposed to unfavourable conditions, i.e., high temperature and relative humidity which accelerate deterioration (Natubhai et al. 2018). These conditions may end up damaging the seed integrity which can result in delayed germination, which is the case for seed lots stored under uncontrolled conditions. Marcos-Filho (2016) further indicated that a uniform metabolic state in seeds that are composed of seed lots with low vigour or low physiological potential is required to reach the same metabolic level and, in the process improving uniformity, synchronisation of the germination and reduce the mean emergence time. Research suggests that storage of seeds at a controlled low temperature, with seed packaging materials and exploitation of seed treatments, such as invigoration, would help in delaying the seed deterioration processes, thereby maintaining the viability and vigour of the seeds during long storage periods (Thirusendura Selvi, Saraswathy 2018). The temperature and relative humidity are a couple of the storage factors that accelerate the seed deterioration and increase the loss of viability (De Vitis 2020), hence maintaining good storage conditions would help to maintain the high seed vigour.

Seed invigoration with $ZnSO_4$ has the potential of reducing the mean germination time and

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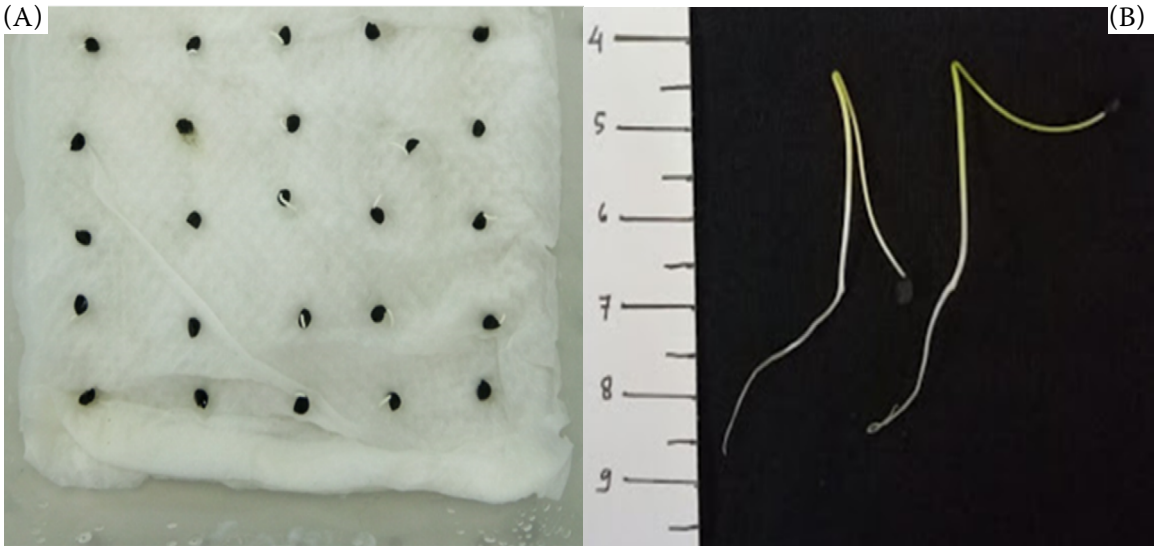

Figure 4. Observation of (A) the radicle emergence and (B) normal seedlings of the TSS germinated at 25 ± 1 °C

improve the germination rate. Previous research showed that seed treatment of other crops, such as barley with $ZnSO_4$, increased the germination and seedling development (Ajouri et al. 2004). The radicle emergence test provides emerging or newly established laboratories with an opportunity to gain early information on the eventual count of normal seedlings. The use of radicle emergence and the mean germination time (MGT) based on the radicle emergence in germination tests were found to be predictive of normal germination in the field in some horticultural crops (Mavi et al. 2016).

The current results indicated that the germination rate increased by 13.2% and the germination percentage increased by 45.4% due to the seed invigoration with 0.5% $ZnSO_4$ which was higher when compared to the non-invigorated seeds. These results concur with Ozturk et al. (2006) who found that the zinc in newly developed radicles and the coleoptiles during seed germination were higher > 200 mg/kg which indicates the involvement of zinc in physiological processes during early seedling development. $ZnSO_4$ has been linked to protein synthesis, membrane function, cell elongation and resistance to abiotic stress (Cakmak 2000), these could be assumed to have contributed to early the radicle emergence which eventually resulted in the reduction in the time for the RE observation and MGT.

The findings further support the earlier work on wheat (*Triticum aestivum*) (Amin et al. 2016) and rice (*Oryza sativa*) (Lee, Kim 2000), where an improved germination rate and percentage in seeds subjected to seed invigoration for 24 hours were reported. Seed invigoration with other osmotic solutions, such as seed priming with GA3, osmoconditioning with PEG-6000 (Dursan, Ekinci 2010) and hydropriming with distilled water for 24 hours, significantly reduced the germination time and improved the emergence rate and seedling vigour. However, seed invigoration with $ZnSO_4$ proved to be more effective considering that it took only 10 hours while in previous research seeds were invigorated for 24 hours, but still had similar results.

Seed invigoration resulted in a speed up in the germination both in the laboratory and the field, a reduced number of days to a single count of RE and an improved number of normal germinated seedlings which are an indication that seed invigoration helps in the seed quality improvement. The effect of seed invigoration on the TSS was related to other findings in which the invigorated seed resulted in positive effects on horticultural and field crops (Saglan et al. 2010; Nugrahan et al. 2021). Seed invigoration activates some enzymatic and metabolic processes within the seed without causing seed germination, which later enhances the germination when the seed is planted. In this research, TSS seed lots were invigorated with $ZnSO_4$ solution for 10 hours which proved to be effective in improving the RE in the TSS. However, the seed vigour for seeds that had deteriorated were not improved, this supports the theory of seed deterioration in that the deterioration cannot be reversed (ISTA 2014). However, invigoration treatments have been widely used to reduce the time between the planting date and the seedling establishment

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period (Sujanthiya et al. 2020) and provide uniform plant growth in different plant crops.

CONCLUSION

Invigoration of TSS with $ZnSO_4$ reduced the time for the single count of radicle emergence and increased the germination percentage, vigour index, germination rate and reduced mean germination time. The radicle emergence of the invigorated seed correlated well with other seed vigour parameters for the seed lots stored under uncontrolled conditions, therefore, there is no need to conduct seed treatments for seeds with high vigour. The single count of the RE was able to separate and evaluate the seed vigour of the TSS after being stored for six months under uncontrolled (ambient) conditions. However, research should be conducted to determine the maximum time on which invigorated seed lots should be stored before being sown.

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