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Invigoration Improved Quality and Storability of True Seed of Shallot (*Allium ascalonicum* L.)

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Abstract

Seed invigoration is a pre-planting seed treatment by balancing the seed water potential and stimulating their metabolic activities so that the seeds germinate simultaneously. In this study, invigoration treatments were applied to improve the quality of the true seeds of shallots (TSS) that had started to deteriorate. Invigoration increases the moisture content of the seeds so the seeds need to be re-dried to extend their storability. The aims of the study were to determine the best invigoration treatment and the drying method to maintain the benefit of invigoration, and to prolong TSS storability after invigoration and drying. This research was conducted at the Laboratory of Seed Quality Testing, IPB University, Indonesia, and consisted of three experiments, i.e., invigoration of deteriorating TSS to improve their viability and vigor, drying after invigoration to extend seed storability, and seed storability after invigoration and drying treatments. The first experiment was arranged in completely randomized design with one-factor (invigoration solutions), i.e. control, medium containing 3% KNO_3, 50 ppm GA_3, 0.5 % $ZnSO_4$ or -10 bar PEG6000. The second experiment was conducted using a two-factor completely randomized design, drying temperature (20˚C, 30˚C, and 40˚C), and drying duration (8 hours, 16 hours, 32 hours, and 48 hours). The third experiment was arranged in a nested design with two factors. The first factor was a seed storage condition, i.e., ambient temperature, air-conditioned room, and refrigerator. The second factor was seed packages, i.e., aluminum foil and polypropylene plastic. Non-invigorated seeds were used as control. The studies were conducted on two shallot varieties separately, “Trisula” and “Lokananta”, except in the third experiment which only used “Lokananta”. The results showed that invigoration in 3 % KNO_3 and 50 ppm GA_3 effectively improved the vigor of deteriorating TSS. Drying the seeds at 40˚C for 8 hours was the most effective method to reduce seed moisture content without reducing the benefit of invigoration. Storing the invigorated TSS in an air-conditioned room with aluminum foil packaging, or in a refrigerator with aluminum foil or plastic packaging until 14 weeks maintained the benefit of invigoration. The quality of the invigorated TSS was maintained until 14 weeks in an air-conditioned room with aluminum foil packaging, or in a refrigerator with aluminum.

Keywords: seed drying, seed storage, viability, vigor

Introduction

The true seed of shallot (TSS) is an alternative planting material in shallot cultivation other than bulb seeds. Botanical seeds have advantages over the bulb seeds, including pathogen-free, less expensive, and allow longer-term storage compared to bulb seeds (Askari-Khorasgani and Pessarakli, 2019). Only 3 kg of botanical seeds is required per ha, in contrast to 2 tons of bulb seeds. TSS have no dormancy, and can be stored for more than one year (Sopha et al., 2015, Rosliani et al., 2018). At 6% moisture content, TSS can be stored for up to 12 months at 4-15˚C with relative humidity (RH) of 40-60% (Selvy and Saraswathy, 2017). Physiological and biochemical changes occur during storage, such as decreased germination and vigor index, decreased activity of dehydrogenase and amylase enzymes, and increased cell membrane leakage (Umesh et al., 2014). At a later stage of deterioration, seed quality decreases, indicated by reduced seed viability and vigor (Brar et al., 2019a).

Seed invigoration is generally used as a pre-planting treatment to improve germination and seedling development by balancing the water potential of the seeds and stimulating the metabolic activities so that the seeds germinate simultaneously (Ilyas,

2012). Invigoration on TSS has been studied using various solutions such as KNO_3 (Muruli et al., 2016), GA_3 (Agung and Diara, 2017), $ZnSO_4$ (Saranya et al., 2017; Kamanga et al., 2021), polyethylene glycol (PEG) (Arin et al., 2011). Invigoration by soaking TSS in 0.5 % $ZnSO_4$ for 10 hours increased germination from 53% to 85.5% (Kamanga et al., 2021), while immersion in 3% KNO_3 for 24 hours increased germination from 75% to 81%, increased vigor index and mean germination time (Muruli et al., 2016). Brar et al. (2019b) reported that invigorating one-year-old TSS using 50 ppm GA_3 for 16 hours improved viability (78%), vigor index-I (germination length x seed germination of 1302.8), and field emergence (65.93%). Invigoration treatment increases seed moisture content, therefore the seeds need to be re-dried to extend their storability. Re-drying chili seeds at 35°C and 75% relative humidity for 48 hours after hydropriming (soaking the seeds in distilled water for a certain period of time) maintained the positive effect of hydropriming without affecting the quality of the seeds. However, re-drying seeds at a low temperature (15°C) occurs slowly and causes a decrease or a delay germination (Demir et al., 2005). Sedghi et al. (2012) reported that a temperature of 20˚C - 30˚C is the ideal for drying following seed invigoration of *Calendula officinalis* L. The role of invigoration treatment in extending seed storability is still debated. Butler et al. (2009) and Wood and Hay (2010) reported that invigoration extends the storability of *Digitalis purpurea* and *Rhododendron griersonianum* seeds, while Hussein et al. (2015) and Malek et al. (2019) reported the contrary, invigoration harms seed storability. Butler et al. (2009) reported that *Digitalis purpurea* seeds invigorated with PEG6000 at osmotic pressure of -1 Mpa and dried properly had an extend storability. On the other hand, Hussein et al. (2015) reported that invigorated rice seeds then stored at a temperature of 25˚C harmed seed storability compared to non-invigorated seed. Invigorated rice seeds (priming) only had 20-30% germination after being stored for 210 days at 25°C, as opposed to non-invigorated rice seeds, which maintained the germination up to 80% after being stored for 210 days at 25°C. The results of the previous studies indicated that the effect of invigoration on the seed storability varied with species. Invigoration followed by appropriate drying does not only improve seed viability and vigor but also extends seed storability. This technique would be very useful in managing seed production, especially those seeds which only be produced once a year such as TSS. This study aims to obtain the optimal invigoration treatment and drying methods for TSS, and to determine their storability post-invigoration.

Material and Methods

Location

Our study was conducted from April 2021 to March 2022 at the Seed Quality Testing Laboratory, Leuwikopo Experimental Station (06˚56'S dan 106˚73' E), Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University.

Materials

The materials used were two varieties of TSS, "Trisula" and "Lokananta". Seed of var. "Trisula" was produced by the Brastagi Vegetable Crops Research Station, harvested on August 24, 2018. The TSS was 32 months after harvest (MAH) when was used for this research, during which time the seed was stored in a refrigerator (4±2°C) with polypropylene plastic packaging. Seed of var. "Lokananta" was produced by PT East West Seed Indonesia with an expired date in April 2021, and previously was stored in an air-conditioned room (18±2°C) with aluminum foil packaging. Invigoration was applied using KNO_3, GA_3, $ZnSO_4$, and polyethylene glycol (PEG6000). Aerated invigoration bottles were used for invigoration at 20± 2˚C. Following invigoration, aluminum foil and polypropylene (PP) plastic packaging were used to store seeds. Germination was carried out in germination boxes with 3-ply of filter paper and put in germination room with temperature of 20± 2˚C.

Treatment

Experiment 1. Invigoration to improve viability and vigor

This experiment was arranged using a one-factor completely randomized design consisting of five treatments, namely no treatment (control), KNO_3 at 3% (Muruli et al., 2016), GA_3 at 50 ppm (Brar et al. 2019b), $ZnSO_4$ at 0.5% (Kamanga et al. 2021), and -10 bar PEG 6000 (Arin et al., 2011). PEG 6000 at -10 bar was prepared by mixing 273 g of PEG in 1-L of distilled water at 20˚C (Michel, 1983). A total of 400 grains of TSS each from the "Trisula" and "Lokananta" varieties were soaked in 200 mL of four types of aerated invigoration solutions in plastic bottles until the seeds were completely immersed; all treatments were repeated four times. Each treatment was incubated at 20˚C for 24 hours except for invigoration in $ZnSO_4$, which was only incubated for 10 hours (Saranya et al., 2017). The seeds were air-dried at room conditions (26-32˚C, 61-79% RH) for 5 hours (Kamanga et al., 2021) germinated in a germinator at 20±2˚C.

Experiment 2. Drying of TSS after invigoration to extend storability

The experiment was arranged using a two-factor completely randomized design. The first factor was the drying temperatures: 20˚C, 30˚C, and 40˚C. The second factor was the duration of drying: 8 hours, 16 hours, 32 hours, and 48 hours. The treatment was applied to two varieties, "Trisula" and "Lokananta", separately. The treatment was repeated four times so that there were 48 experimental units for each variety. Four-hundred TSS that have been invigorated using 200mL of KNO_3 at 3% were air-dried at ambient conditions (26-32˚C, RH 61-79%) for 5 hours. The initial moisture content was determined at a temperature of 103˚C for 17 hours. After the initial seed moisture content had been determined, the seeds were dried at 20˚C, 30˚C, or 40˚C with drying duration according to the treatment, then the seeds were germinated at 20± 2˚C.

Experiment 3. The storability of TSS after invigoration and drying

The experiment was arranged using a two-factor nested design. The first factor was the storage condition: 28±2˚C, RH 73±7% (ambient condition); 18±2˚C, RH 61±7% (air-conditioned room); and temperature 8±2˚C, RH 24±8% (refrigerator). The second factor was the type of packaging, aluminum foil and polypropylene (PP) plastic packaging. The experiment was repeated four times so that there were 28 experimental units per observation. Only TSS var. "Lokananta" was used in experiment 3, due to the limited amount of var. "Trisula" seeds for the study. True shallot seeds (4.6 g) that had been invigorated with 1.6L KNO_3 at 3% for 24 hours were air-dried at ambient condition (26-32˚C, 61-79% RH) for 5 hours, followed by an oven drying at 40˚C for 16 hours. After the seeds reaching the moisture content safe for storage, then were packaged according to the treatments. Seed quality testing was carried out every two weeks for 14 weeks.

Seed Germination and Seedling Growth Measurements

Measurement of seed viability and vigor was based on germination percentage (GP), vigor index (VI), germination speed (GS), radicle emergence (RE), T50, electrical conductivity (EC), mean emergence time (MET), and field emergence (FE).

Germination percentage (GP) was conducted using 50 seeds on 3-ply filter paper and repeated four times in a standard germinator at a temperature of 20± 2˚C. Observations and measurements were made on day 6 and day 12. The criteria for normal seedlings are those having long and straight roots, having long and green cotyledons, and forming an angle in the middle (ISTA, 2018). GP is calculated using the formula:

$$\text{GP (\%)} = \frac{\text{Number of NS I} + \text{Number of NS II}}{\text{Total seeds germinated}} \text{ x } 100\%$$

Where:
NS I: Normal seedlings on the first count (day 6)
NS II: Normal seedlings on the second count (day 12)

Vigor index (VI) was obtained based on the percentage of normal germination on the first count (day 6) of the germination test. VI is calculated using the formula below:

$$\text{VI (\%)} = \frac{\text{Number of normal seedlings on the first count}}{\text{Total seeds germinated}} \text{ x } 100\%$$

Germination speed (GS) was measured every day for 12 days by counting the number of normal seedlings and the difference in hours of each observation (%NS/etmal). SP is calculated using the formula:

$$\text{GS (\%NS/etmal)} = \sum_0^t \left(\frac{\%NS}{etmal}\right)$$

where:
t : observation time
%NS : percentage of normal germination at each observation time
etmal : observation time every 24 hours

Radicle emergence (RE) was measured using 50 seeds per experimental unit on filter paper and repeated four times. Radicle emergence was measured 72 hours after germination (Kamanga et al., 2021), when a radicle has emerged at least 2 mm (ISTA, 2018).

T50 value is measured based on the time required to reach the percentage of normal germination of 50% of the total GP, and is expressed in days. Measurements were carried out every day for 12 days. T50 is calculated by the formula of Coolbear et al. (1984):

$$T_{50} \text{ (days)} = ti + \frac{(n50\% - ni)}{(nj - ni)} (tj - ti)$$

where:
n 50% is the number of germinated seeds (50% of the total germinate), nj and ni are the cumulative number of seed germinated by adjacent counts at times tj (day) and ti (day), respectively, when ni < n50% < nj.

Electrical conductivity (EC) was measured by immersing 50 TSS grains into 50 mL of distilled water

for 24 hours according to method of Dias et al. (2006) and covered with aluminum foil. Each experimental unit was replicated four times. EC is calculated using the formula:

$$EC\ (\mu S.cm^{-1}.g^{-1}) = \frac{\text{Conductivity of sample} - \text{blank}\ ((\mu S.cm^{-1}.g^{-1})}{\text{Seed weight(g)}}$$

Mean emergence time (MET) is the number of days required for the seedlings to emerge in the field. MET was carried out every day until 14 DAP. MET was calculated based on the number of seedlings that appeared with a hypocotyl length of at least 2 cm per day which was recorded every day until 14 DAP.

$$\text{Mean emergence time} = \frac{\Sigma\ (nxt)}{\text{Number of normal seedlings}}$$

Where:
n : Number of seeds that appear at –t
t : Days after planting (DAT)

Field emergence (FE) is the percentage of seeds that grow into normal seedlings in the field. Observations were made on 14th days after planting (DAT). Seedlings are considered to be normal if the hypocotyl that appears above the soil surface has reached a minimum of 2 cm.

Data Analysis

Analysis of variance was carried out using Statistical Analysis System (SAS) version 9.1.2 at α=0.05; Tukey test was used for further analysis. Correlation between simple linear regression was performed on the measured variables.

Result and Discussion

Invigoration to Improve Viability and Vigor

Invigoration treatments increased the vigor of TSS var. "Trisula", and increased the viability and vigor of var. "Lokananta" (Table 1). Invigoration in 50 ppm GA_3 or 3% KNO_3 increased the vigor of var. "Trisula", as indicated by the increase in vigor index of 71.5% and 64.9%, and germination speed of 15.0 % NS/etmal and 15.1 % NS/etmal, respectively. However, the invigoration did not increase seed viability based on the germination percentage (Table 1). Similar results were reported by Muruli et al. (2016) in that GA_3 at 50 ppm increased the highest vigor index-I (seed germination x germination length) (from 911 to 1342) compared to other invigoration media, while KNO_3 at 3% accelerated germination time from 5.6 days to 4.6 days. Selvarani and Umarani (2011) also reported that invigoration of TSS with KNO_3 at 3 % increased germination speed from 20.5 % NS/etmal to 25.9 % NS/etmal. Invigoration using PEG6000 at -10 bar in var. "Lokananta" resulted in the highest germination percentage (96.50 %) which was not significantly different from other invigoration treatments. Invigoration using PEG6000 at -10 bar increased the activity of α-amylase enzymes and antioxidant enzymes such as superoxide dismutase, catalase, and peroxidase (Yuan et al., 2010, Sheteiwy et al., 2016). The increase activities of the α-amylase enzyme in seeds accelerated food mobility and increased seed viability (Yuan et al., 2010). On the other hand, KNO_3 at 3% increased the TSS vigor of var. "Lokananta" better than other invigoration treatments, as shown by the highest germination speed (20.28% NS/etmal) and the lowest T_{50} of 4.07 days. It is suspected that the nitrate content within KNO_3 enhances the synthesis of the nitrate reductase and antioxidant enzymes such as superoxide dismutase, catalase, and ascorbate peroxidase, which play important roles in increasing the speed of germination (Lara et al., 2014).

In addition to increasing viability and vigor, invigoration reduced membrane leakage as indicated by the lower electric conductivity (Matthews et al., 2018). In TSS "Trisula", invigoration using GA_3 at 50 ppm resulted in the lowest electric conductivity of 9.54 $\mu S.cm^{-1}.g^{-1}$, which was not significantly different from the other invigoration treatments, while in "Lokananta", treatment with 0.5% $ZnSO_4$ resulted in the lowest electric conductivity (9.54 $\mu S\ .cm^{-1}.g^{-1}$) which was not significantly different from PEG6000 at -10 bar (Table 1). The results of this study was in line with the results obtained by Brar et al. (2019) in which invigoration of TSS with GA_3 at 50 ppm, KNO_3 at 0.5%, Thiram at 2 $g.kg^{-1}$, and KH_2PO_4 at 0.5% significantly reduced cell membrane leakage, as indicated by their electric conductivity values. Amooaghaie et al. (2010) reported that hydropriming and osmo-priming treatments could increase the stability of tomato seed cell membranes by up to 23%. According to Siri et al. (2013) increased cell membrane stability was associated with an increase in antioxidant enzymes during invigoration. The increase in total antioxidant activity, dehydro-ascorbate, and catalase in invigorated seeds, promoted the defense mechanism in protecting cell membranes. According to Orhan et al. (2011), antioxidants can protect cell membranes from the damaging effects of ROS, such as single oxygen, superoxide, peroxyl, and hydroxyl.

"Trisula" and "Lokananta" demonstrated different responses to invigoration treatments. "Trisula" seeds used in this study were 32 month after harvest, which could have reached the critical period, the third period in seed life time (Sadjad, 1993), during which time the seed vigor declines more rapidly than the viability. The "Lokananta" seeds had just expired but

is possible to be re-labelled if germination percentage is at least 70% (Table 1). Based on vigor index and electric conductivity, the increase in "Trisula" vigor was higher than "Lokananta", while the germination speed showed the opposite. These data indicated that invigoration treatment was more effective when applied to seeds with low vigor than those with high vigor. The difference in the effectiveness of invigoration is in line with the research results of Muruli et al. (2016) and Brar et al. (2019b) that the seed quality improvement in response to invigoration was higher in seeds with low vigor than high vigor.

Invigoration did not increase mean emergence time and field emergence; in "Trisula" the mean emergence time was 8-10 days and it was 8-9 days in "Lokananta" (Table 2). Field emergence of the "Trisula" and "Lokananta" was 21-35% and 24-52%, respectively, except for the "Trisula" treated with PEG6000 at -10 bar which only had 7% field emergence due to high fungal attacks.

Correlation analysis between variables in "Trisula" showed that VI, GS, and RE had a negative and significant correlation with MET, but the correlation

Table 1. Quality of TSS in response to invigoration treatments

Treatments	GP (%)	VI (%)	GS (%NS/etmal)	T_{50} (days)	RE (%)	EC ($\mu S.cm^{-1}.g^{-1}$)
			"Trisula"			
Control	80.50 a	35.00 b	11.90 b	6.45 a	51.50 a	178.85a
PEG6000 -10bar	78.50 a	46.50 ab	12.70 ab	6.31 a	56.00 a	110.94b
3% KNO_3	76.50 a	64.00 a	15.10 a	5.03 a	73.50 a	96.03b
50 ppm GA_3	77.50 a	71.50 a	15.00 a	5.03 a	78.50 a	91.02b
0.5% $ZnSO_4$	76.00 a	49.00 ab	12.58 ab	5.72 a	76.00 a	91.87b
F-test	ns	**	**	*	ns	**
CV (%)	6.67	16.14	13.28	15.57	14.74	14.97
			"Lokananta"			
Control	84.00 b	57.50 b	14.05 c	5.73 a	92.50 a	113.46 a
PEG6000 -10bar	96.50 a	79.00 a	19.13 ab	4.59 bc	89.00 a	67.44 bc
3% KNO_3	88.50 ab	86.50 a	20.28 a	4.07 c	92.50 a	79.39 b
50 ppm GA_3	94.50 ab	79.00 a	17.48 ab	5.08 b	94.50 a	75.28 b
0.5% $ZnSO_4$	93.00 ab	77.50 ab	17.18 b	5.03 b	90.50 a	57.56 c
F-test	*	**	**	**	ns	**
CV (%)	7.15	17.25	13.96	12.52	5.65	12.58

Note: Values within the same column followed by the same letter are not significantly different based on the Tukey test at α=0.05. * = significant at P < 0.05, ** = significant at P < 0.01, ns = not significantly different. GP = germination percentage, VI = vigor index, GS = germination speed, RE = radicle emergence, EC = electric conductivity.

Table 2. Mean emergence time and field emergence of TSS in response to invigoration treatments

Treatments	"Trisula"		"Lokananta"	
	MET (days)	FE (%)	MET (days)	FE (%)
Control	9.71 ab	34.50 a	8.75 ab	34.00 a
PEG6000 -10bar	10.00 a	7.00 b	8.72 ab	30.00 a
3% KNO_3	8.90 b	35.00 a	8.14 b	51.50 a
50 ppm GA_3	8.70 b	21.00 a	8.93 ab	31.50 a
0.5% $ZnSO_4$	9.09 ab	29.00 a	9.11 a	24.00 a
F-test	*	**	ns	ns
CV (%)	7.29	30.80	5.76	24.17

Note: Values within the same column followed by the same letter are not significantly different based on Tukey's test at α=0.05, * = significant at P < 0.05, ** = significant at P < 0.01, ns = not significantly different. MET = mean emergence time up to 14 DAP, FE = field emergence at 14 DAP.

was not significant on FE (Table 3). This indicated that the higher the VI, GS, and RE values in "Trisula", the faster the time for seedlings to emerge in the field, although the correlation between the two was not very strong (-0.47 to -0.53). Meanwhile, T_{50} was significantly and positively correlated with MET, which indicated that the higher the T_{50} value, the longer the time to seed emergence in the field.

TSS Drying to Extend TSS Storability

Post-invigoration seed drying is a critical step in maintaining the positive effect of invigoration. Inappropriate post-invigoration drying of seeds can cause seed decline or even death due to the deterioration process (Farooq et al., 2018). Seed drying needs to be conducted until a safe moisture content of 8% had been reached (Ministry of Agriculture, 2017). Drying the seeds at a temperature of 20°C and 30°C for up to 48 hours could not reach the required moisture content whereas at 40°C for 8 hours could reach an MC <8% (Table 4).

In general, the drying temperature did not affect the viability and vigor of "Trisula" (Table 5). Although the drying temperature had a significant effect on VI, it did not show a clear pattern of influence, while drying time affected viability and vigor shown by GP and VI. Drying time of 8 to 32 hours did not affect GP and VI, but drying for 48 hours decreased GP and VI (Table 5). These data indicate that drying at 40˚C is relatively safe as long as the drying does not exceed 32 hours. According to Farooq et al. (2010), the decrease in the seed quality due to drying for too long occurred because of the decrease in α-amylase and dehydrogenase enzymes activities, leading to a decrease in metabolic activities of the seeds.

There were differences in the initial seed quality of "Trisula" and "Lokananta". The age of "Trisula" seeds was 32 MAH, so the seeds might have entered a critical period of seed storage, whereas "Lokananta" seeds had expired. The drying temperature did not affect viability and vigor as shown by GP and GS (Table 6). However, the length of drying duration as a

Table 3. Linear regression between seed quality testing in the laboratory and seedling emergence time in the field of "Trisula" and "Lokananta" varieties

Parameters	"Trisula"			"Lokananta"		
	r	R^2	Equation	r	R^2	Equation
			Mean emergence time			
RE	-0.470*	0.208	y = -0.018x + 10.50	0.128tn	0.016	y = 0.012x + 7.560
VI	-0.531*	0.279	y = -0.022x + 10.46	-0.191tn	0.036	y = -0.007x + 9.307
GP	0.039tn	0.001	y = 0.005x + 8.866	0.267tn	0.071	y = 0.021x + 6.809
GS	-0.509*	0.215	y = -0.175x + 11.64	-0.275tn	0.075	y = -0.057x + 9.753
T_{50}	0.568*	0.276	y = 0.401x + 6.987	0.382tn	0.146	y = 0.321x + 7.158
			Field emergence			
RE	0.131tn	0.017	y = 0.098x + 18.7	-0.252tn	0.063	y = -0.757x + 103.7
VI	0.045tn	0.002	y = 0.036x + 23.38	0.179tn	0.032	y = 0.213x + 17.99
GP	0.115tn	0.013	y = 0.284x + 3.201	-0.327tn	0.107	y = -0.781x + 105.5
GS	0.169tn	0.027	y = 1.180x + 9.403	0.151tn	0.022	y = 0.958x + 17.31
T_{50}	-0.170tn	0.028	y = -2.437x + 39.21	-0.295tn	0.087	y = -7.508x + 70.97

Note: * = significantly different according to the Pearson correlation test. RE = radicle emergence, VI = vigor index, GP = germination percentage, GS = germination speed.

Table 4. Decreases in the moisture content (%) of TSS "Trisula" and "Lokananta" after invigoration and drying at different temperatures and duration

Drying duration (hours)	"Trisula" Drying temperature			"Lokananta" Drying temperature		
	20°C	30°C	40°C	20°C	30°C	40°C
8	10.7	12.9	7.2	9.8	22.3	6.4
16	10.9	11.2	7.2	5.3	9.3	6.6
32	9.2	11.7	6.4	4.2	11.0	6.2
48	9.2	10.7	6.3	3.6	10.3	5.2

Table 5. Germination percentage, vigor index, germination speed, and T50 of TSS of "Trisula" variety as a response to temperature and drying time post-invigoration.

Drying temperature (˚C)	GP (%)	VI (%)	GS (%NS/etmal)	T_{50} (day)
20	66.00 a	35.50 b	11.39 a	6.22 a
30	68.88 a	46.63 a	11.77 a	6.22 a
40	68.13 a	35.25 b	10.97 a	6.34 a
F-test	ns	**	ns	ns
CV (%)	10.23	20.82	9.64	5.45
Drying duration (h)				
8	68.83 a	42.50 a	11.08 a	6.26 a
16	69.00 a	41.50 a	11.62 a	6.36 a
32	74.17 a	42.83 a	11.56 a	6.16 a
48	58.67 b	28.33 b	11.24 a	6.27 a
F-test	**	**	ns	ns
CV (%)	10.23	20.82	9.64	5.45

Note: the values within the same column followed by the same letter are not significantly different based on Tukey's test at α =0.05, ** = significant at P < 0.01, ns = not significantly different. GP = germination percentage, VI = vigor index, GS = germination speed.

Table 6. Germination percentage and speed of germination of TSS "Lokananta" in respond to drying temperature and duration of post-invigoration seeds

Treatments	GP (%)	GS (%NS/etmal)
Drying temperature (˚C)		
20	83.38 a	13.84 a
30	83.50 a	13.36 a
40	87.75 a	13.58 a
F-test	*	ns
CV (%)	6.41	9.19
Drying duration (h)		
8	89.67 a	14.87 a
16	89.00 ab	14.08 a
32	83.33 bc	13.53 a
48	77.50 c	11.89 b
F-test	**	**
CV (%)	6.41	9.19

Note: The values within the same column followed by the same letter are not significantly different based on Tukey's test at α =0.05, * = significant at P < 0.05, ** = significant at P < 0.01, ns = not significantly different. GP = germination percentage, GS = germination speed.

single factor or its interaction with drying temperature, affected the viability and vigor as shown by GP, GS, VI and T50 (Tables 6 and 7). In general, drying up to 32 hours did not reduce the seed viability and vigor of post-invigoration. At low temperatures (20˚C) prolonged drying (48 hours) reduced seed quality. It is suspected that a deterioration process occurs because of the high moisture content. These data indicate that the TSS quality post-invigoration can be maintained by drying for 8-32 hours. However, longer drying duration will risk reducing the seed quality, so drying for 8 hours at 40˚C, which can reduce the MC by 8%, is the best drying method.

Table 7. Vigor index and T50 of "Lokananta" variety in respond to the interaction between temperature and drying duration post-invigoration

Drying duration (h)	VI (%)			T_{50} (days)		
	20˚C	30˚C	40˚C	20˚C	30˚C	40˚C
8	67.00 a	74.00 a	71.50 a	5.55 a	5.67 a	5.65 a
16	60.00 ab	73.50 a	66.50 a	5.89 ab	6.09 ab	5.83 ab
32	60.50 ab	41.00 bcd	53.50 abc	6.02 ab	6.67 bc	6.26 abc
48	41.50 bcd	19.00 d	34.50 cd	7.67 d	7.11 cd	6.96 cd
F-test		**			*	
CV (%)		18.24			5.53	

Note: The values within the same column followed by the same letter are not significantly different based on Tukey's test at α =0.05, * = significant at P < 0.05, ** = significant at P < 0.01. VI = vigor index.

The TSS Storability After Invigoration and Drying

The quality of the dried TSS post-invigoration can be maintained for 14 weeks after storage (WAS), as indicated by vigor parameters such as RE, VI, and GS (Figure 1). This result is in line with the research of Srinivasan and Saxena (2001) which showed that the improvement of radish seed quality after invigoration using PEG6000 -0.75 Mpa could be maintained for 10 months of storage. Basra et al. (2003) showed that canola seeds invigorated using PEG10000 for 4 hours and then stored in plastic packaging (PP) at 8˚C for 6 months resulted in better GP than control. During storage, the viability of invigorated "Lokananta" was similar to that of non-invigorated TSS (Figure 1B). This is in line with the results of an experiment I which

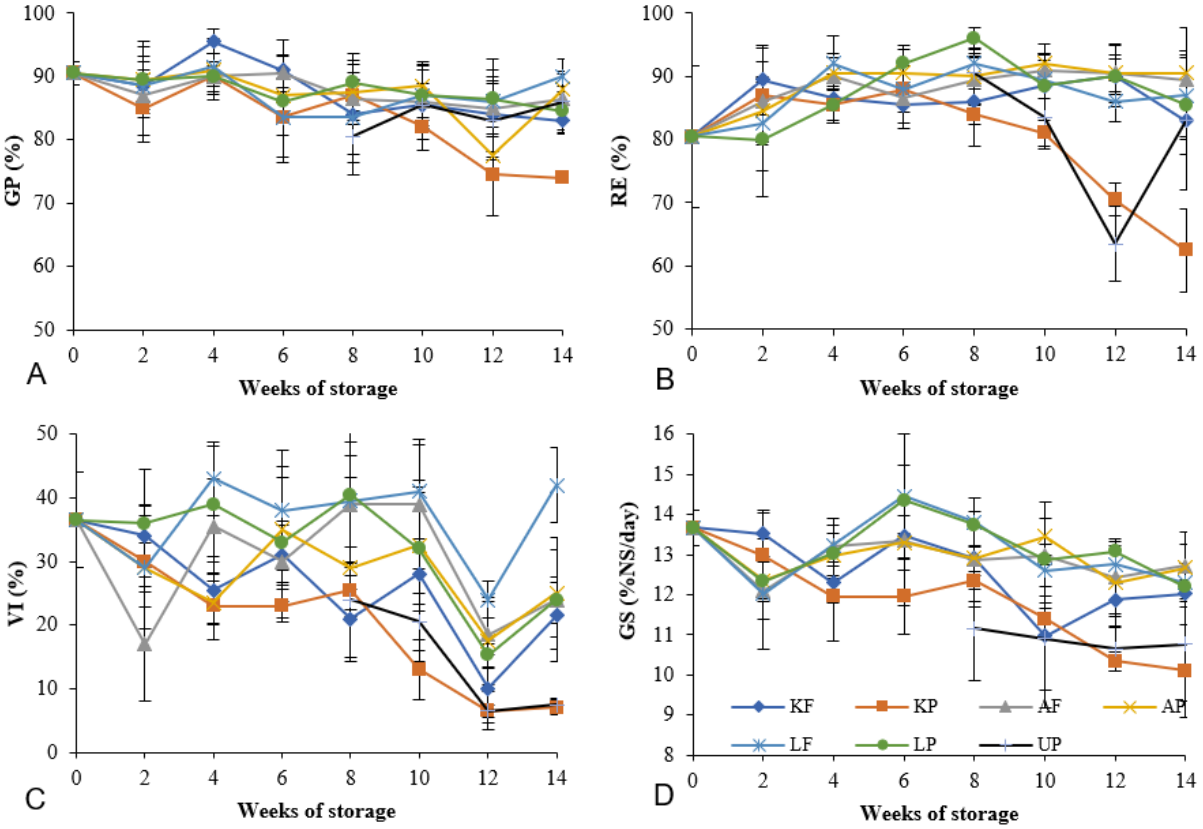

Figure 1. Quality of true shallot seeds "Lokananta" after invigoration for 14 weeks after storage at different storage condition and packaging material. GP = germination percentage, RE = radicle emergence, VI = vigor index, GS = germination speed. KF = room conditions and aluminum foil; KP = room conditions and PP plastic; AF = AC and aluminum foil chamber; AP = AC room and PP plastic; LF = refrigerator and aluminum foil, LP = refrigerator and PP plastic; UP = without invigoration.

showed that invigoration of the “Lokananta” with 3% KNO_3 for 24 hours was more effective in increasing seeds vigor than viability. The positive effect of invigoration during storage can be maintained if the seeds are stored in proper conditions. Invigorated TSS stored in a refrigerator or air-conditioned room using aluminum foil or plastic packaging can maintain the positive effect of invigoration better than invigorated TSS stored in room conditions (Figure 1). The positive effect of invigoration during storage will gradually disappear if stored under room conditions. Invigorated TSS stored at room conditions had decreased vigor quality since the eighth week. The quality of invigorated TSS, stored at room conditions using plastic packaging declined faster than those packaged using aluminum foil. The decreased vigor of invigorated TSS stored at room temperature could be caused by high temperature and high relative humidity (RH) (28±2ºC, 73±7%),which lead to the accumulation of reactive oxygen species (ROS), loss of membrane integrity, and depleted seed reserve, and resulted in seed damage (Liu et al. 2016). Seed storage at high RH could also increase lipid peroxidation and seed deterioration (Wang et al., 2018).

Butler et al. (2009) and Wood and Hay (2010) had reported that invigoration can extend the storability of seeds. However, Hussein et al. (2015) and Malek et al. (2019) reported the contrary, invigoration can negatively affect seed storability. The response of seed storability to invigoration treatment is influenced by the initial vigor of the seed (Powell et al., 2000). Invigoration can adversely affect seeds with high initial vigor, while invigoration treatment can extend the storability of seeds on seeds with low initial vigor. In this study, invigoration could improve TSS quality as indicated by improvement in seed viability and vigor (Tables 1 and 2). After storage in the various environment for 14 weeks, the invigorated TSS demonstrated better seed quality than the non-invigorated TSS (Figure 1). Referring to Powel et al. (2000) the positive effect of invigoration can be maintained during storage for TSS with low initial vigor (35-57.5%). Thus, invigoration could be used as seed pretreatment to enhance field emergence and to prolong the storage, even though long storage post-invigoration is not recommended.

Our study had demonstrated that invigoration treatment with KNO_3 at 3%, drying at 40°C for 8 hours followed by storage in a refrigerator with aluminum foil packaging can improve the quality and storability of TSS. This results would be useful to manage the availability of quality TSS which can only be produced once a year.

Conclusion

Seed invigoration in medium containing 3 % KNO_3 and 50 ppm GA_3 increased seed vigor, did not affect seed viability of the true shallot seed “Trisula”, but increased seed vigor and viability of “Lokananta”. True shallot seeds with lower vigor is more responsive to the invigoration treatment than those with high vigor. Drying at 40˚C for 8 hours is effective to reduce seed moisture post invigoration and to maintain seed quality after invigoration. The quality of TSS post-invigoration can be maintained for 14 weeks after storage in an air-conditioned room (18±2°C, RH 61±7%) using aluminum foil packaging, or in refrigerator (8±2°C, RH 24±8%) using aluminum foil or plastic packaging.

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