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Evaluation of Suitable Germination Media and Germination Test Procedure for Shallot *(Allium cepa L. aggregatum)* Seed

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Abstract: The experiment was conducted in the laboratory at Debre Zeit Agricultural Research Center in two phases with the objectives of to identify the best germination media to conduct proper germination and to optimize germination test procedure for shallot seed. In the first phase the experiment consisted of 6 treatments of three germination media (Top of paper, Between paper and sand) and two shallot varieties (DZSHT-91-2B and DZSHT-157-1B). The second experiment consisted of 27 treatments as three water application times (24 hours, 48 hours and 72 hour), three germination papers (filter paper, grade paper and kitchen paper), and three amount of water to be applied (2.5ml, 5ml and 7.5ml of distilled water). The treatments were arranged in both phases in factorial combination with complete randomized design (CRD) in four replications. The analysis of variance showed significant variation for shallot seed germination on different germination media**.** The highest mean values were obtained on DZSHT-91-2B on Top of Paper (86%) and the lowest mean value was recorded for DZSHT-157-1B on sand (60.5%). In addition highest mean value of speed of germination was obtained from top of Paper (7.9), and the lowest mean value was observed from shallot seed sown in between Paper (4.5). Therefore top of paper is the best germination media as compared to sand and in between paper to have the maximum germination for shallot seed. With regard to optimization of germination test procedure as both the main effect and the interaction effect of the seed physiological quality parameters indicated that the highest mean values were recorded when shallot seed was planted on Kitchen paper with 5ml water application once for twenty four hour for three times during the whole germination period.

Keywords: Shallot, Germination Paper, Seed Quality, Optimization, Germination Media

1. Introduction

Shallot (*Allium cepa L. aggregatum*) is widely cultivated and favorite vegetable crop in Ethiopia. Seed germination test is one of the parameters of seed quality. Shallot and onion belong to the genus *Allium.* Shallot is formerly known as *Allium ascalonicum* L., a separate species from onion, but later it was identified that shallots are *Allium cepa* var. *ascalonicum* Backer as the most important subgroup of the Aggregatum group and the only one grown commercially [5, 11]. The genus *Allium* L. includes more than 700 species, which wildly grow in the temperate, semiarid and arid regions of the northern hemisphere and s hallots are known as vegetative propagated varieties of *Allium cepa*. True seed production potential of shallot was tested in many countries of the World [9, 6].

Onion and shallot are among the most important vegetable crops grown around the world mainly for its requirements in the daily consumption and for its health benefits for human beings, The Allium (Onion, Shallot, and Garlic) groups are produced in a scattered manner in different parts of Ethiopia. In Ethiopia, lack of improved varieties and absence of any responsible agents who produces and distributes the improved seed materials to the growers were the main challenges at the country level. However, Cultivation of high-quality true shallot seeds (TSS) under suitable environmental condition with good agricultural management can be possible. The production of bulb shallot is restricted to highland areas under rain-fed conditions. Seed is considered as an integral part of agriculture since it provides the

maximum limit of crop yield of all other production inputs [14]. In shallot particularly, the seed is considered as a very significant input since farmers have to invest about 25-50% of total cost to acquire it [8]. It is one of the most important cash crops and traditionally produced under rain-fed conditions in many regions of the country (Hararghe, Shoa, Arsi, Bale, Gojjam.) by small holder farmers for consumption and income generation. It has grown mainly for its bulbs even if the green shoots are used for salads and other purposes [9, 2, 13].

Likewise the demand for quality onion seed is increasing, However, seed supply is inadequate, its price is increasing every year and onion seed available in the market are poor in quality [1, 10]. Onion seeds are poor in keeping quality and lose viability within a year. Owing to these challenges, onion seed production gradually started by smallholder farmers in different parts of the country.

Allium cepa L. is cultivated as a biennial but some types have treated as a perennial crop for its seed productions [4]. For the purpose of onion bulb production, the crop has produced as annuals either by seeds or by bulb methods.

According to Tabor (2018), the use of bulbs as planting material is not advisable, because bulbs are expensive, bulky to transport, transmit diseases from one generation to the next, and have short shelf life [12]. As a result, production of shallot decreased considerably in favor of the seed propagated onions. Three shallot accessions were identified (DZSHT-91-2B, DZSHT-193-1A and DZSHT-157-1B), that could bolt and produce seeds.

According to ISTA (2014), Different germination media for shallot seeds were identified as top of paper, between paper and sand, even though, it has not clear laboratory procedure which provides the maximum germination [7]. Therefore this research activity was designed to identify the best germination media to conduct proper germination for shallot seed and to optimize germination test procedure for shallot seed.

2. Materials and Methods

The experiment was conducted at Debre Zeit Agricultural Research Center in two phases.

First experiment

The first experiment consisted of 6 treatments as factorial combination as three germination media (Top of paper, Between paper and sand), two shallot varieties (DZSHT-91-2B and DZSHT-157-1B). Shallot seed sample of 2020 crop season harvest was used.

The second experiment: optimization of Shallot seed germination test procedures

This experiment consisted of 27 treatments as factorial combination of three water application times (24 hours, 48 hours and 72 hour), three germination papers (filter paper, grade paper and kitchen paper), and three amount of water to be applied (2.5ml, 5ml and 7.5ml of distilled water). The treatments were arranged in complete randomized design (CRD) with four replications.

In this experiment 'top of paper method and glass petri dishes with a diameter of 120mm was used by sterilized and wiping with 70–95% alcohol. The germination papers were cut and lined in appropriate way on the Petri dish. The working sample of 400 shallot seeds were counted randomly and 100 Shallot seeds were spread uniformly on the surface of the paper with each peter dishes in four replications. The petri dishes were covered and placed in the seed germinator with optimum temperature with the range of 20°-30°C and distilled water was applied on the determined time. Speed of germination counting and scoring was started five days after planting and continued until the 12^{th} days and percent germination was determined based on normal seedlings.

Finally all physiological seed quality parameters were measured and recorded. The average percentage is calculated to the nearest whole number. Normal seedlings were identified and dried for 24 hr at 80°C, and weighed after drying. The average dry weight per seedling is an estimate of relative seedling vigor. Seedling length measurement was conducted after 12^{th} days of planting based on ISTA (2014) [7]. Ten seedlings were randomly measured and the mean values of root and shoot length was recorded.

2.1. Data Collection

At the end of germination test, percentage of normal seedlings (NS%), abnormal seedlings (AS%), fresh un germinated seeds (FUGS%), dead seed (DS%), Speed of Germination (SPG), Standard Germination (SDG%), Seedling Shoot Length (SSL), Seedling Root Length (SRL), Seedling Dry Weight (SDW), Vigor Index VI and Vigor Index VII were calculated and recorded.

2.2. Data Analysis

Data analysis was carried out by SAS software, version 9.0 (SAS, 2002) package with one way analysis of variance to determine the degree of relationship among physiological parameters. The mean comparison was done using Minimum Significant Difference (HSD) using Tukey test at 5% level of significance.

3. Results and Discussions

3.1. Standard Germination

The analysis of variance showed significant variation for shallot seed germination on different germination media. The highest mean values were obtained on DZSHT-91-2B on top of Paper (86%) followed by DZSHT-157-1B on top of Paper (74.5%), and the lowest mean value was recorded for DZSHT-157-1B on Sand (60.5%) (table 1). Even though, there is significant difference among the treatments in this experiment, all values were meet the national seed standard set for shallot seed minimum germination (table 1).

Table 1. *Mean values of seed physiological parameters of shallot.*

Treatments	Parameters								
	Abnormal seedling (%)	Dead seed (%)	Standard germination	Shoot length	Root length	Seedling dry weight	Vigour index one	Vigour index 2	Speed of germination
DZSHT-91-2B Top of Paper	8.0c	6d	86a	10.7a	4.5a	0.17a	1313.4a	14.6a	7.9a
DZSHT-91-2B Between Paper	15.0b	15ac	70bc	9.4b	3.9ac	0.14ac	941c	9.7bc	5.4c
DZSHT-157-1B top of Paper	13.5b	12c	74.5b	9.7ab	4.2ab	0.15ab	1038.6b	11.5b	6.05b
DZSHT-157-1B Between Paper	17ab	13.5bc	69.5bc	9.1b	3.7bd	0.13bc	897.6c	9.2bd	4.5d
DZSHT-91-2B Sand	18ab	16.5ab	65.5cd	9b	3.5cd	0.12c	821.9d	8.1cd	5.5bc
DZSHT-157-1B Sand	21a	18.5a	60.5d	8.6b	3.2d	0.12c	716.4e	7.4d	4.6d
CV	14.8	11.8	3.6	5.9	6.6	8.5	3.3	10.2	4.3
HSD	5.2	3.7	5.8	1.28	0.59	0.02	73.2	2.3	0.57
Fvalue									

CV= Coefficient of variation.

3.2. Speed of Germination

Speed of germination is one of the indicators used for assessing the vigor of seeds and seeds that have high germination speed were found vigorous in the field and could be escaped harsh conditions. The analysis of variance indicated that the main effect of suitable germination media for shallot seed highly significantly ($P \leq 0.001$) affected the speed of germination (Annex table 7). The highest mean value of speed of germination was obtained when Shallot seed was planted on top of Paper (7.9), and the lowest mean value was observed from shallot seed sown in Between Paper (4.5) and on Sand (4.6) (table 1).

Therefore from this experiment Top of paper is the best media for shallot seed germination in the laboratory as compared to the others. After identification of the suitable media optimization of germination test procedure is important to verify the germination procedure.

The following phase of the experimental approach was optimization of germination procedure which was conducted based on the methodology indicated below.

3.3. Evaluation of Germination Papers on Speed of Germination

The analysis of variance indicated that the main effect of germination papers, water application times amount of water application and the interaction effects significantly ($P \leq 0.01$) affected the speed of germination (Annex table 8). The highest mean value of speed of germination was obtained when shallot seed planted on kitchen paper (10.4) followed by grade Paper (10.3) and the lowest mean value was observed from shallot seed sown on Filter paper (10.18) (table 2).

Table 2. *Mean value of shallot seed physiological quality under different germination paper.*

Treatment	Parameters						
	Standard germination	Seedling shoot length	Seedling root length	Seedling dry weight	Vigour index one	Vigour index two	Speed of germination
Filter paper	81.05b	3.6b	2.3b	0.035a	488.15c	2.93a	10.18b
Grade paper	82.22b	3.69b	2.55a	0.03b	524.9b	2.60b	10.3a
Kitchen paper	83.66a	4.027a	2.38b	0.03b	529.3a	2.72ab	10.4a
CV	2.5	3.3	4.5	14	0.4	13.3	3.5
HSD	1.18	0.07	0.6	0.003	1.28	0.207	0.206
F value	19.20	65.22	42.90	32.75	5134.92	42.57	17.96

CV= Coefficient of variation, HSD= Minimum Significant Difference.

The Water application times at 24 hour gave the highest mean values for all seed physiological quality parameters as (10.57) for speed of germination and (84) for standard germination. (table 3).

Table 3. *Mean values of seed physiological quality parameters under different water application time.*

Treatment	Parameters						
	Standard germination	Seedling Shoot Length	Seedling Root Length	Seedling Dry Weight	Vigour Index one	Vigour Index Two	Speed of Germination
24 hours	84.9a	3.73b	2.7a	0.34a	549.3a	3.43a	10.57a
48 hours	81.8b	3.89a	2.35b	0.032b	513.4b	2.67b	10.45a
72 hours	80.1c	3.73b	2.24c	0.202c	479.5c	2.16c	9.9b
CV	2.5	3.3	4.5	14	0.4	13.3	3.5
HSD	1.18	0.07	0.6	0.003	1.28	0.207	0.206
F value	19.20	65.22	42.90	32.75	5134.92	42.57	17.96

CV= Coefficient of variation, HSD= Minimum Significant Difference.

3.4. Evaluation of Water Application Time on Standard Germination

The analysis of variance showed significant variation for Shallot seed germination at 5% level of significance ($p \leq 0.001$) for amount of water to be added and the highest mean value was recorded at 5 ml water amount and the lowest value is at 2.5 ml. Highest mean value was also recorded at 5ml water application as compared to 2.5ml and 7.5 ml water level.

Table 4. *Mean values of shallot seed physiological quality under different water level.*

Treatment	Parameters						
Water level	**Standard germination**	**Seedling Shoot Length**	**Seedling Root Length**	**Seedling Dry Weight**	**Vigor Index one**	**Vigor Index index two**	**Speed of Germination**
2.5ml	78c	3.19b	2.13c	0.02c	414.70b	1.27c	9.48c
5ml	86a	4.05a	2.49b	0.05a	563.60a	3.80a	11.18a
7.5 ml	82.9b	4.11a	2.67a	0.038b	564.08a	3.15b	10.30b
CV	2.5	3.30	4.50	14.00	0.40	13.30	3.50
HSD	1.18	0.07	0.60	0.003	1.28	0.207	0.20
F value	19.20	65.22	42.90	32.75	5134.92	42.57	17.96

CV= Coefficient of variation, HSD= Minimum Significant Difference.

3.5. Seed Vigour

Seed vigour is a single concept reflecting several characters determines the seed quality and uniform emergence potential of plants in field under variable range of environments [3]. In this experiment the analysis of variance showed significant variation for shallot seed vigour on different germination papers, Water application times and amount of water application ($p \leq 0.001$). As indicated in the above table the maximum mean value for seedling vigour both vigour index I and vigour index II was observed on kitchen paper at 5ml water level with twenty four hour water application time interval. (tables 3 and 4).

3.6. Interaction Effect on Seed Physiological Quality

According to the three way interaction mean result of germination media, water application time and amount of water level; the highest mean value for most of the seed physiological parameters was recorded at the interaction of kitchen paper with twenty four water application time at 5ml water level three times during the germination period (table 5) gave the highest mean value for standard germination (89) and also for other seed physiological parameters.

Table 1. *Mean values of the three way interaction effect of Shallot seed physiological quality parameters.*

No	Treatment			Parameters						
	Paper	**Water level**	**Time**	**Standard germination**	**Seedling Shoot Length**	**Seedling Root Length**	**Seedling Dry Weight**	**Vigor Index one**	**Vigor Index index two**	**Speed of Germination**
1	Filter Paper	2.5ml	24	82c-g	2.97hi	2.21eg	0.02e-g	424.93no	1.64k-m	9.87f-l
2	Filter Paper	5ml	24	87abc	4.25b-d	2.82ab	0.05a	615.1c	4.77a	11.02a-e
3	Filter Paper	7.5ml	24	81d-g	3.26fgh	3.00a	0.04ab	507.4j	3.64c-g	10.35e-h
4	Filter Paper	2.5ml	48	79e-h	2.95hi	2.13fg	0.01fg	401.82qr	1.38lm	9.35i-l
5	Filter Paper	5ml	48	83.5a-f	4.15bcd	2.27d-g	0.04ab	536.1h	3.96a-e	11.45abc
6	Filter Paper	7.5ml	48	77gh	4.21bcd	2.32def	0.04abc	503.15j	3.27c-h	9.90ef-k
7	Filter Paper	2.5ml	72	79e-h	2.73i	2.28d-g	0.01fg	396.67r	1.19lm	8.9l
8	Filter Paper	5ml	72	83b-f	4.07cd	2.13fg	0.04ab	514.61i	3.73-f	10.67b-g
9	Filter Paper	7.5ml	72	78fgh	7.07cd	2.25d-g	0.03bcd	493.58k	2.82f-i	9.62h-l
10	Grade paper	2.5ml	24	81d-g	3.11gh	2.15fg	0.02efg	426.4n	1.62klm	10.00f-j
11	Grade paper	5ml	24	88ab	4.07cd	3.05a	0.05a	626.8b	4.83a	11.42a-d
12	Grade paper	7.5ml	24	87a-c	4.02d	2.97ab	0.47ab	608.92cd	4.02a-d	10.52c-h
13	Grade paper	2.5ml	48	78f-h	3.25f-h	2.12fg	0.01fg	419.1-n	1.36lm	9.3i-l
14	Grade paper	5ml	48	85.5a-d	4.08cd	2.9ab	0.03bcd	596.72e	2.98e-i	11.57ab
15	Grade paper	7.5ml	48	86.5a-d	4.07cd	2.83ab	0.03bcd	597.63e	3.02def-i	10.45d-h
16	Grade paper	2.5ml	72	74.5hi	3.33e-g	2.1fg	0.01g	404.6q	0.74m	9kl
17	Grade paper	5ml	72	85.5a-d	3.21fgh	2.12fg	0.03cde	456.2l	2.67g-j	10.57c-h
18	Grade paper	7.5ml	72	87abc	4.07cd	2.68bc	0.02d-f	588.27f	2.17ijkl	10.65b-g
19	Kitchen Paper	2.5ml	24	84a-e	3.18gh	2.07fg	0.02efg	441.33m	1.68j-m	10.1e-i
20	Kitchen Paper	5ml	24	89a	4.45ab	3.08a	0.05a	670.53b	4.67ab	11.02a-e
21	Kitchen Paper	7.5ml	24	85.5a-d	4.27b-d	3.01a	0.47ab	623.08a	4.05a-c	10.85b-f
22	Kitchen Paper	2.5ml	48	74hi	3.55ef	2.11fg	0.01fg	418.69p	1.1m	9.7ghijkl
23	Kitchen Paper	5ml	48	84.5a-e	4.04cd	2.07fg	0.03b-d	398.95qr	2.96e-i	11.04a-e
24	Kitchen Paper	7.5ml	48	85.5a-d	4.62a	2.49c-e	0.03b-d	608.74d	2.99e-i	10.52c-h
25	Kitchen Paper	2.5ml	72	70.5i	3.62e	2.03fg	0.01g	517.15i	0.7m	9.09j-l
26	Kitchen Paper	5ml	72	84.5a-e	4.04cd	2.07fg	0.03bcd	398.95qr	2.96e-i	11.04a-e
27	Kitchen Paper	7.5ml	72	79e-h	4.38a-c	2.52cd	0.03c-e	546g	2.44h-k	9.88ef-l

No	Treatment			Parameters						
	Paper	Water level	Time	Standard germination	Seedling Shoot Length	Seedling Root Length	Seedling Dry Weight	Vigor Index one	Vigor Index index two	Speed of Germination
	CV			2.5	3.3	4.5	14	0.4	13.3	3.5
	HSD			1.18	0.07	0.6	0.003	1.28	0.207	0.206
	F value			19.20	65.22	42.90	32.75	5134.92	42.57	17.96

CV= Coefficient of variation, HSD= Minimum Significant Difference.

3.7. Correlation Analysis

The result of correlation analysis show a positive and significant (P<0.001) correlation between Parameters, standard germination (SDG) strongly correlated with Speed of germination, vigor index I vigor index II, seedling shoot length, root length, seedling dry weight. The other seed physiological parameters are also strongly and positively correlated with each other (table 5).

Table 2. *Pearson's simple combined correlation coefficients of seed physiological quality parameters of shallot.*

Variable	Standard germination	Seedling Shoot Length	Seedling Root Length	Seedling Dry Weight	Vigor Index one	Vigor Index index two	Speed of Germination
SDG	1	0.35***	0.45***	0.58***	0.72***	0.65***	0.74***
SHL		1	0.44***	0.67***	0.82***	0.67***	0.54***
RL			1	0.6***	0.78***	0.61***	0.39***
SDW				1	0.79***	0.99***	0.69***
VI					1	0.82***	0.70***
VII						1	0.72***
SPG							1

*, **, ***, Significantly correlated at P < 0.05, P < 0.01, P < 0.001 respectively and P>0.05 "ns" non-significant.

Table 3. *ANOVA for shallot seed germination test done under different germination med.*

Source	DF	Mean squares for the measured variables								
		ASD	DS	SDG	SHL	RL	SDW	VI	VII	SG
Trt	5	79.3***	75.7***	304.8***	2.4**	0.8***	0.001**	170881.9****	27.4***	6.4***
Rep	3	5.5ns	1.9ns	5.7ns	0.2ns	0.1ns	0.0001ns	399.4ns	0.9ns	0.6ns
Error	15	5.2	2.6	6.5	0.3	0.06	0.0001	10167	1.08	0.06
corrected total	23									
CV		14.8	11.8	3.6	5.9	6.6	8.5	3.3	10.2	4.3
R2		84	90	93	71	81	76	98	89	97
G-mean		15.4	13.5	71	9.4	3.8	0.14	954.8	10.16	5.6

*, **, ***, significant effect at P < 0.05, P < 0.01, P < 0.001 respectively and P>0.05 "ns" non-significant,.

Table 4. *ANOVA for laboratory parameters of optimization of Shallot seed germination test procedures.*

Source	DF	Mean squares for the measured variables						
		SDG	SHL	RL	SDW	VI	VII	SPG
Media*hours*water	20	36.5***	0.34***	0.23***	0.00005***	5739.1***	0.4***	0.35***
Media	2	61.5***	1.6***	0.33***	0.0002***	18418.9***	1***	1**
Hours	2	215***	0.29***	2.09***	0.001***	43897***	14.8***	4.1***
Water	2	586***	9.5***	2.7***	0.008***	266913***	63.7***	26***
Rep	3	1.9ns	0.007ns	0.018ns	0.000003ns	1.3ns	0.1ns	0.12ns
Error		4.4	0.01	0.01	0.0001	5	0.1	0.1
Corrected Total		107	-					
CV		2.5	3.3	4.5	14	0.4	13.3	3.5
R^2		87	96	94	92	99	94	86
G –mean		82	3.7	2.4	0.03	514	2.8	10.3
F value		19.20	65.22	42.90	32.75	5134.92	42.57	17.96

*, **, ***, significant effect at P < 0.05, P < 0.01, P < 0.001 respectively and P>0.05 "ns" non-significant.

4. Conclusions

Onion and shallot are among the most important vegetable crops grown around the world mainly for its requirements in the daily consumption and for its health benefits for human beings, The Allium (Onion, Shallot, and Garlic) groups are produced in a scattered manner in different parts of Ethiopia.

In Ethiopia, lack of improved varieties and absence of any responsible agents who produces and distributes the improved seed materials to the growers were the main challenges at the country level. However, Cultivation of high-quality true shallot seeds (TSS) under suitable environmental condition with good agricultural management

can be possible. The production of bulb shallot is restricted to highland areas under rain-fed conditions. It is one of the most important cash crops and traditionally produced under rain-fed conditions in many regions of the country (Hararghe, Shoa, Arsi, Bale, Gojjam.) by small holder farmers for consumption and income generation. It has grown mainly for its bulbs even if the green shoots are used for salads and other purposes.

According to ISTA (2014), different germination media for shallot seeds were identified as top of paper, between paper and sand, even though, it has not clear laboratory procedure which provides the maximum germination result for shallot seed [7]. Therefore identification of the suitable media for optimum germination and optimizing the procedure has to be important with research evidence for the users.

From this experiment as indicated in the result on top of paper is the suitable media which gave the maximum germination as compared to germinating shallot seed on sand and in between paper and also Kitchen paper with the application of 5ml water at 24 hours interval for 3 times during the entire germination period is the appropriate and optimum germination procedure for shallot seed.

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