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Report

Effect of Different Grow out Test on the Seedling Anatomy and Germination Attributes of Faba Bean (*vicia faba* L.)

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Abstract

Ability to geminate is very important attributes of seed quality. Critical factors for germination include moisture, oxygen, temperature and light. In combination with the factor's germination media and place to layout are very important. Germination test was conducted on Ashebeka variety of Faba bean. The aim of the study was to test the effect of different germination Condition on seedling anatomy and germination potential of the seed. Germination room (GR) with air conditioning, Germination chamber with Adjustable (Relative humidity, Temperature and light) and Normal room were used. 50seeds/germination box with four replication were panted using CRD design in each room. Germinated seed and its shoot length counting was started to recorded starting from after five days within 3 day interval (5^{th}, 8^{th}, 11^{th} and 14^{th}) of planting to observe effect of growing room on germination and it attributes. Finally, data collected was analyzed using Statistical software. The mean squares of each treatment reveal that there is a significant variation on germination and seedling attributes for all counting days due to growing room. Among treatment Germination room with air condition shows best performance both quantative and qualitative data. While germinating seed in growing chamber leads to increment of seedling/germinated seed due to mutation or another factor. Germinating seed under normal room also affect the situation due to temperature fluctuation.

Keywords

Anatomy, Seedling, Mutation, Temperature, Light

1. Introduction

Quality seed is the key for development of agricultural activity [1]. Faba beans germinate with the cotyledon remaining below ground (hypogeal germination). The epicotyl (part of the stem above cotyledons) grows toward soil surface and pushes the main shoot (plumule) above ground [2]. As the shoot grows towards the soil and emerges, growing points (nodes) become evident. The first two nodes with faba beans develop below or at soil surface and the small leaves associated at these are called scale leaves. Regrowth is possible from buds at the base of these scale leaves [3]. Radicle emerges from the base of the embryo axis and is the growing point of the root, and the plumule, the growing point of shoot, is at the upper end of the embryo axis, above the cotyledons [4]. A series of programmed cell divisions after fertilization, and differentiation results in the development of the miniature seed that undergoes several molecular, cellular and metabolic changes to mature into a fully functional and viable seed [5]. A quality seedling must be above 80% normal germination for

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faba bean [6]. A typical young seedling consists of three main parts: the radicle (embryonic root), the hypocotyl (embryonic shoot), and the cotyledons (seed leaves). Providing the optimum environment can decrease germination time, improve uniformity and increase the number of seeds that germinate [7]. This study was conducted to evaluate room and method that suit for germination and other seed ling attributes. The main objective is to identify the germination suit method and condusive room.

2. Materials and Method

2.1. Description of the Study Area

The study was conducted at Kulumsa Agricultural Research Center (KARC) from 01-14 days of April, 2024 under laboratory conditions. The site is located at 8 ̊00' N and 39 ̊07' E at an elevation of 2210 m above sea level in Arsi Administrative Zone of Oromia Regional State, 167 km South East of Addis Ababa. The Agro-climatic condition of the area is wet and receives a unimodal mean annual rainfall of 809.15 mm from March to September; however, the peak season is from July to August. The maximum and minimum mean temperature is 23.08 and 9.9 ℃, respectively [8].

Methods

Hundred seeds of from pre basic seed of Faba bean were planted in different growth room to see the effect of germinating seed under different condition. Three different growing conditions were used (Seed germination room with Air conditioning (Ac) having a temperature adjusted to 18.5 with normal electric light. The same experiment conducted in other room using Germination chamber with adjustable relative humidity, light and temperature. The 3rd the same experiment is conducted under Normal room at room temperature and relative humidity.

Data collected

Both quantative data (morphology and anatomy) Seedling and Qualitative data was taken for this work.

2.2. Seed Germination Data

Germination test allows seed producer to determine and compare the quality of a seed lot before it is planted. This information can be used to satisfy labeling laws and provide for standardized marketing of seed. The normal germinated seed lings after 14 days of planting was calculated excluding Dead (un germinated seed) and Abnormal seed ling at final count then percentage is calculated as follows.

$$\text{Seed germination (\%)} = \frac{\text{Number of normal seed ling(\#)} * 100}{\text{Total Planted seeds (\#)}}$$

During Germination test only normally, germinated seedling was counted as Germinated seed while dead seed (not germinated) and abnormal seed ling were not included according to ISTA seed germination procedure.

Shoot and Root length:

At the time of germination count, 10 normal seedlings were taken at random. The length between the collar and tip of the primary root was measured as root length and the mean length expressed in centimeter. From the 10 seedlings used for measuring the root length, the length between collar and tip of the primary shoot was measured as shoot length and the mean value expressed in centimeter. Most primary growth occurs at the apices, or tips, of stems and roots. Primary growth is a result of rapidly dividing cells in the apical meristems at the shoot tip and root tip [9]. Subsequent cell elongation also contributes to primary growth. The growth of shoots and roots during primary growth enables plants to continuously seek water (roots) or sunlight (shoots).

Leaf length: The length was measured from the apex of the blade to the base of the petiole, and width was measured at the widest point perpendicular to the longitudinal axis of the leaf.

Leaf width: Even though leaf width is influenced by water availability, light intensity, temperature, and climate variables such as latitude and altitude, It is measured at the middle for each using cm [10].

Table 1. *Conditions of the germination room.*

Treatment	The condition of the room			
	Temperatures	Relative humidity	Light condition	Seed Germinator putted in/on
Germination Room (GR)	18.5	60-63	Normal electric (ampule light)	putted on germination stalk/stand
Growth chamber (GC)	20.5	80-100	Seed Germinator light	putted in Seed germination chamber
Normal Room (NR)	20-23	60-67	Normal room light	Arranged on flat table

Seedling Anatomy

Seedling anatomy and morphology is important to decide seedling normality [11].

There are three main parts of a leaf – Leaf base, leaf lamina, and petiole. There are two different types of leaves – simples leaves and compound leaves [12]. The other types of leaves include acicular, linear, lanceolate, orbicular, elliptical, oblique, centric cordate, et.

Table 2. *Anatomical description of seedling.*

#	Anatomical description	Treatments			
	Seedling anatomy	**Germination Room**	**Germination chamber**	**Normal Room**	**Remark**
1	Leaf scale	Visible	less visible	visible	D/t in color
2	Length of internode	2-3cm	4-7cm	2-4cm	vary
3	smoothness of seedling stem	Relatively smooth	White less smooth	Green smooth	vary
4	straightness of seed ling	Moderate	Very zigzag	Straight	Vary
5	Overall seedling color	Green	Gray to white	Deep green	vary
6	Total number of leaf/stems	3-4 (pairs)	3-4(pairs)	3-4(pairs)	Similar
7	Total number of nodes/stems	4-5	4-5	4-5	Similar
#	Leaf anatomy				
1	Shape	rounded or oval	Not well opened	rounded	Ovate
2	Openness	Opened	Not well opened	opened	vary
3	Tip of leaf apex	Bluse	obtuse	bluse	Vary
4	Veins	Visible	Not well identified	visible	Vary
5	Midribs of leaf	Relatively thick	Thin	thick	Vary
6	Base	Truncate	cordate	Truncate	Vary
7	Length of the petiole (cm)	2-3(cm)	2-4(cm)	2-3(cm)	vary
8	Stipulate	Visible	Not well identified	Visible	vary
9	Leaf length (cm)	3-4(cm)	1-2(cm)	3-4(cm)	Vary
10	Width of the leaf at middle (cm)	1.5-2(cm)	1-1.5 (cm)	1.5-2(cm)	Vary

The leaf apex can exhibit various shapes and characteristics depending on the species, including pointed, rounded, acuminate, or truncate. While the leaf blade is primarily involved in capturing light and facilitating gas exchange, the leaf apex plays a role in protecting the delicate vascular tissues and regulating water loss through transpiration. Additionally, the shape and structure of the leaf apex can influence the overall aerodynamics and mechanical stability of the leaf, particularly in response to environmental factors such as wind, rain, and herbivory [13]. Germination and seed survival increased in seeds stored at higher relative humidity and led to earlier germination [14]. The number of seedlings and tree diameter was strongly correlated with the relative light intensity [15]. Seed germination and seedling attributes are affected by method of sowing and environmental factors [16].

3. Results and Discussion

Table 3. Analysis of variance (ANOVA) results.

Quality parameters	Source of variation and mean squares					
	Treatments (df=2)	Error (df=9)	Grand Mean	F-value	P-value	Coff. Var. 5%
Germination on 5th day	588.583**	1.833	76.167	321.05	0	1.78
Germination on 8th day	588.563**	1.834	81.167	411.89	0	1.67
Germination on 11th day	21.3333**	1.5556	94.333	13.71	0.09	1.32
Germination on 14th day	36.75**	1.1944	94.75	30.77	0.0001	1.15
Shoot length on 5th day	48.0158**	0.8908	7.9417	53.9	0	11.88
Shoot length on 8th day	191.396*	3.514	18.917	54.47	0.2142	9.91
Shoot length on 11th day	191.396**	3.514	18.917	37.37	0.001	9.91
Shoot length on 11th day	355.646^{ns}	3.882	29.708	91.62	0.827	6.63
Root length on 14th day	621.064**	5.814	49.678	106.82	0.6232	4.85
Total seedling length on 14th day	621.064**	5.814	49.678	106.82	0.0016	4.85

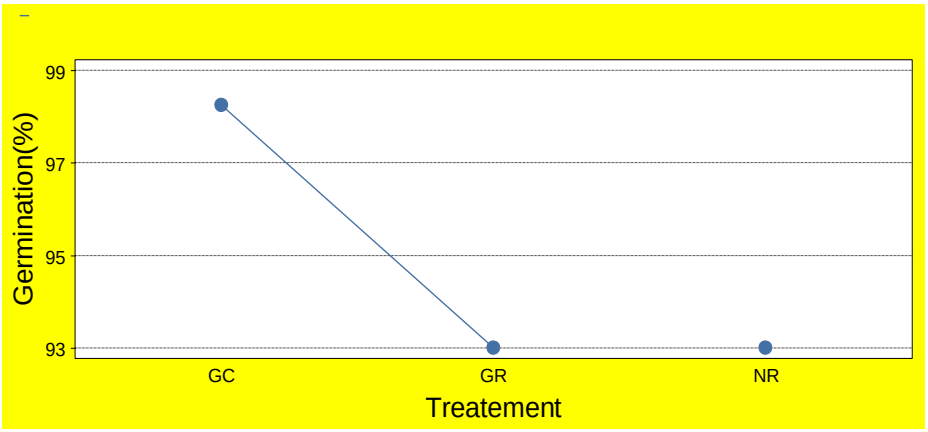
**=Highly significant variation at $\alpha_{5\%}$

There was a highly significant variation among treatments for a number of seed germinated on 5^{th}, 11^{th} and 14^{th} days after planting while it is significant variations observed on 8^{th} day. This could be due to variation of factors like temperature, light intensity and relative humidity. In agreement with this temperature can influence the radicle, plumule and seedling length. In faba beans, alternate primary branches (‘tillers’) usually originate from the base just above ground level (usually 1–5 primary branches on the main stem, depending on variety and growing conditions).

Table 4. Mean separation for each treatment.

#	Treatments	GD5	GD8	GD11	GD14	SHLD5	SHLD8	SHLD11	SHLD14	RLD14	TSLD14
1	GC	88.5^{a}	93.5^{a}	97^{a}	98.25^{a}	11.7^{a}	26.625^{a}	30.375^{a}	39.87^{a}	23.925^{a}	63.8^{a}
2	GR	75.75^{b}	80.75^{b}	93^{b}	93^{b}	7.25b	16.875^{b}	26.625^{a}	28^{b}	18.982^{b}	45^{b}
3	NR	64.25^{c}	69.25^{c}	93^{b}	93^{b}	4.875c	13.25^{c}	24.75^{a}	21.25^{c}	17^{c}	40.233^{c}
Alpha		0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Standard Error		0.9574	0.957	0.8819	0.7728	0.05	1.3255	4.3978	1.3932	0.5582	1.705
Critical Value at (5%)		2.1659	2.166	1.995	1.7482	2.262	2.9985	9.9484	3.1516	1.2628	3.857

Germiation Room: All Quality parametres like shootlength, Rootlength of the seeling, germintion %, Total seedlength and anatomical parts were optimum enough to use this room for conduct germination due to relative humidity, Light utility and temprature control is adjustable.



GC=Germination chamber=Germination room, NR=Normal room

Figure 1. *Mean germination of each treatment on final count days.*

Figure 2. *Pictorial view of seed germinated in Germination room.*

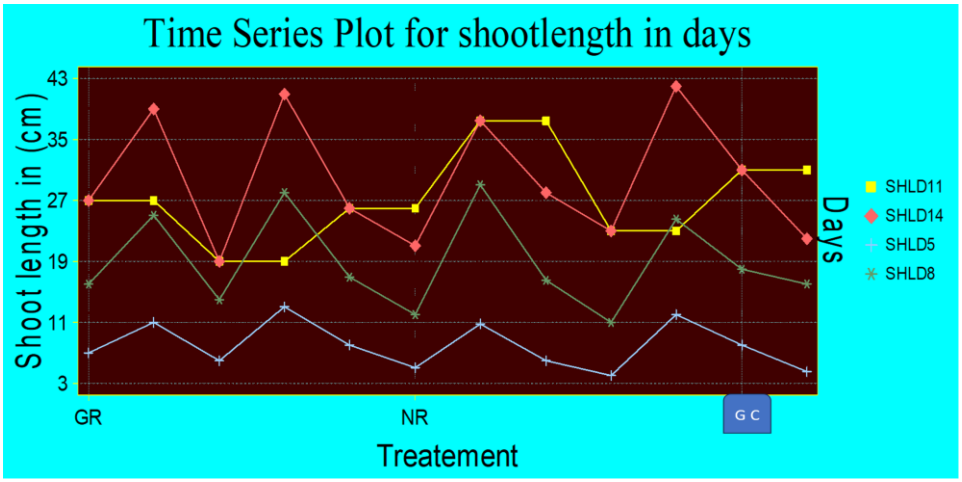


Figure 3. *Increment of seed length in days.*

Germination chamber:

A germination chamber is an enclosed area, where the temperature and humidity can be regulated. There is often some sort of tray stacking system where it is easy to see which trays have germinated and be able to easily insert and remove trays.

Mutation" is the source of most genetic variation and the motor of evolution. It is a natural process, which occurs spontaneously and slowly over generations in people, plants, animals and all living beings.

Figure 4. *Seed germinated in GC.*

Seedling sample from each treatment were compared for each parameter.

Figure 5. *Seedling Germinated at different room.*

GR=Germination room, NR=Normal room, GR=Germination chamber.

As we have mentioned on table 2 all Anatomical part of seedling were observed by using light illuminating work board under laboratory just to identify the variation and variation was observed.

4. Summary and Conclusion of the Report

Conducting seed germination under controlled temperature and relative humidity using Normal electric light improve the overall seedling attributes and germination quality. From the experiment there was a significant variability for quantative traits. The variability of seedling anatomy has been also visually observed for different qualitative traits.

But seed germination using seed germination chamber affect the normality of seedling by making seedling shoot parts more giant and whiter in color up down structure. Beside this it can also leads to wrong counting of normal seed germination due to most of the seeds were bearing three and more seedlings. This could be due to the growth chamber uses its own light that result in mutation/three and more seedling/seed is sprouted or further investigation needed to explain the reason more.

Abbreviations

GC	Germination Chamber
GR	Germination Room
NR	Normal Room

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Author Contributions

Megersa Bayisa Debelo is the sole author. The author read and approved the final manuscript.

Conflicts of Interest

The author declares no conflicts of interest.

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