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Screening of Some Accesions Of Field Pea (*Psium sativaum* L.) For Salt Tolerance at Eastern Ethiopia.

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ABSTARCT

Field Pea (*Pisumsativum* L.) is a pulse crop that belongs to the family Leguminosae. In Ethiopia, salt-affected soils are prevalent in the Rift Valley and the lowlands. This problem is expected to be severe in the years to come. Exploiting genetic variability of field pea accessions that can cope with such saline environment is vital. The objective of the study was to assess salt tolerance of field pea accessions during germination and seedling stage, as well as maturity. Twenty field pea accessions and four NaCl (0, 50, 75 and 100mM) concentrations were selected for laboratory experiment. The following parameters such as, germination rate, germination percentage, seedling shoot length, seedling root length, seedling shoot fresh weight, seedling root fresh weight, seedling shoot dry weight and seedling root dry weight under the laboratory experiment. It is found that salt stress significantly decreased almost all parameters of field pea accessions. The extent of decrease varied with accessions and salt concentrations. Most accessions were highly susceptible to 100mM NaCl concentration. The correlation analysis revealed positive and negative highly significant association among these parameters. Accessions 231214, 230048, 240706 and 202282 were better salt tolerant. These accessions could be used for the development of field pea accessions for improved yield under salt stress condition.

Keywords: Field Pea; Pisumsativum L. Salinity; Accessions; NaCl; Germination;

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INTRODUCTION

Salinity is one of the world's most serious environmental problems in agriculture. The increasing world population, especially in arid and semi-arid regions, food shortages and land scarcity are compelling the use of lands not utilized because of salinity and other soil stress. In the arid and semi-arid parts of Africa, for instance, salinity and alkalinity are major problems affecting about 24% of the continent [1]. According to Eswaran*et al*. [2] about 30% of the population of Africa or about 250 million people are living on or are dependent on this type of land. African countries like Kenya (8.2 Mha), Nigeria (5.6 Mha), Sudan (4.8 Mha), Tunisia (1.8 Mha), Tanzania (1.7 Mha) and Ghana (0.79) have salt affected areas of various degrees (FAO, 2000). Salinity exerts its undesirable effects through osmotic inhibition and ionic toxicity [3]. Increased salinity caused a significant reduction in germination percentage, germination rate, and root and shoots length and root and shoots fresh weights [4].

In Ethiopia, salt-affected soils are prevalent in the Rift Valley and the lowlands [5]. In addition to that in Ethiopia 44 Mha (36% of the country's total land areas) are potentially susceptible to salinity problems. According to out of the 44 Mha,33Mha have dominantly salinity problems, 8 Mha have combined salinity and alkalinity problems, salt affected soils increased from 6% to 16%of the total land area of Ethiopia[5] .For example, soil salinity has caused a significant abandonment of banana plantation and showed a dramatic spread to the adjacent cotton plantation of MelkaSadi Farm [6].HailayTsige *et al.*[7] also indicates that of the entire Abaya State Farm, 30% has already been salt affected. This problem is expected to be severe in the years to come. This is because under the prevailing situation of the country, there is a tendency to introduce and implement large-scale irrigation agriculture so as to meet the demands of the ever- increasing human population by elevating productivity[8]. In the absence of efficient ways of irrigated water management, salt builds up is an inevitable problem. To alleviate this problem it can be done by using either physical or biological practice.

Field Pea (*PisumsativumL.*), isa pulse crop and belongs to the family Leguminosae, and contains seven pairs of chromosome. It is one of the world's oldest domesticated crops [9]. In Ethiopia field pea is a highly consumed pulse in the daily diet of the society in urban and rural areas. It is eaten whole, split or milled usually fresh, fried, boiled or mixed with other cereals to make various types of Stews and Soups [10]. Little information has been reported about the effect of salt on germination, seedling growth and yield and yield related traits in field pea growing in Ethiopia. In addition to this, most of the small-scale farmers are not aware of the status of their soils [11]. In view of the above problem, the present study was to assess the phenotypic variation of some accession of field pea through laboratory screening under salt stress conditions.

MATERIALS AND METHODS

Seeds of twenty accessions of field pea were obtained from Institute of Biodiversity Conservation (IBC). The description of the accessions was given in the Table1.The accessions were collected from different parts of Ethiopia. The laboratory experiment was conducted in the Department of Biology, Genetics Laboratory, Haramaya University, Ethiopia.

Laboratory Experiment

In order to investigate the response of the twenty field pea accessions to NaCl salinity, seeds of pea accessions were first sterilized in 5% sodium hypochlorite solution for 10 min and then thoroughly rinsed with distilled water. Germination experiment was conducted in a laboratory, at room temperature using the procedures followed by [12]. Prior to experiment petri dishes were thoroughly washed and sterilized in hot air oven at 70 ^{0}C for 36 hours and what man filter paper for 24 hours at 70 ^{0}C[13].The petri dishes were lined with what manpaper and arranged in completely randomized design (CRD) in factorial combination with three replications. Germination test was carried out at four concentration levels (0, 50, 75,100mM) by adding 2.93, 4.39 and5.85g of NaCl [14]. Each petridish was supplied with 10ml of the respective treatment solution and on alternate days[15] .No salt was added to the control. Ten seeds were applied per dish. Then the seeds were checked for germination every other day and the germination count was continued until the 15th day. A seed was considered to have germinated when both the plumule and radical emerged $\geq$0.5 cm.

Germination and seedling related traits

Germination rate: the average number of days needed for plumule or radical emergence was calculated according [16].

$$\text{Germination rate} = \frac{NT3+NT6+NT9+NT12+NT15}{\text{Total number seed germinated}}$$

Where T= number of seeds germinated at day 3, 6,9,12 and 15.
N= days (3, 6,9,12 and 15)

Germination percentage: the germination percentage was calculated using the formula below for each replication of the treatment after fifteen days of germination [17].

$$\text{Germination percentage} = \frac{\text{Total number of seeds germinated}}{\text{Total Number of seeds planted}} \times 100$$

Seedling shoot length: it was obtained at the 15thday the mean shoot lengths of five randomly picked seedlings from each petri dish in centimeters using draftsman ruler.

Seedling root length: was also obtained at the 15th day by measuring root lengths of the five randomly picked seedlings from each petri dish in centimeters using draftsman ruler and the average was recorded.

Seedling shoot-to-root ratio:was calculated as the ratio of seedling shoot length to seedling root length.

Seedling shoot fresh weight: itwas measured by recording the average weight of shoots of five randomly picked seedlings from each petri dish in gram using sensitive balance.

Seedling root fresh weight: it was also measured in grams by weighting the mass of roots of five randomly picked seedlings from each petri dish using sensitive balance and the average was recorded.

Seedling shoot dry weight: wastaken as the means of five randomly pickedseedlings of each petri dish after oven-drying, at 70^{0}C for 72 hours and weighting them using sensitive balance.

Seedling root dry weight: itwas also recorded as the average of five randomly picked seedlings of each petri dish of thereplication after oven-drying at 70^{0}C for 72 hours.

Ranking accessions for salt tolerance:a scoring system was used to rank the accessions after the laboratory screening test for their tolerance to salinity. The aggregated score was computed as it was recommended by Fawzi*et al.*(2002) formula:

$$\text{Score} = G_{0.0} \times L_{0.0} + 2\,(G_{50} \times L_{50}) + 3(G_{75} \times L_{75}) + 4(G_{100} \times L_{100})$$

Where G_i is the number of germinated seeds,
L_i is the average length of the shoots of the germinated seeds, and
i (0, 50, 75,100mM) is the concentration levels.

The highest score obtained was ranked as number 1; the second highest score rank number 2, and so on.Based on this criterion, eight accessions with high ranks were used for the subsequent greenhouse experiment for the evaluation of the effect of salinity on yield and yield related parameters.

Data Analysis

The data obtained from laboratory experiment were analyzed statistically for analysis of variances using SAS (version 9.1) statistical software (SAS Institute Inc., USA). Whenever treatment differences were significant, means were separated using the least significant differences (LSD) test at 5% level. Correlation analysis was employed in order to study the associations of one trait with the others.

RESULT AND DISCUSSION

Effect of salinity on germination rate of field pea accessions

As shown in table 2, germination rate varied among the accessions; it started on the 3rd days and completed on the 15th day in different accessions. At the control, accession243843 (3.10) followed by accession 230048 (3.22) took the shortest germination rate whereas accessions 243848, 32499, 32052 and 32001 had the longest germination time with a value greater than 5. On the other hand, accession 231214 followed by 243842 recorded the shortest germination time with 3.96 and 3.99 at 50mM NaCl respectively whereas, accession 243848 had delayed germination rate with 10.75. Furthermore; at 75mM salinity level, accession 231214 (4.81) followed by accession 202282 (4.85) had shown fastest germination rate but, the other accessions had germination rates ranging from 5-13.50. Similarly, at 100mM salinity level accessions 231214 followed by 240706 had shown the fastest germination rate with 5.23 and 5.69whereas the other accessions had germination rates of 6-14. Accessions with higher values germination rates in all salinity levels are considered as salt sensitive while, accessions with lower values germination rates are considered as salt tolerant. This means that salinity caused prolongation of the time to germination.

Table 1: Description of field pea accessions that were used for the study

S.No	Accessions	Region	Zone	Woreda	Latitude	Longitude	Altitude
1	32001	Oromiya	MISRAK SHEWA	ADA'A CHUKALA	08-41-00-N	38-53-00-E	1950
2	32052	Oromiya	BALE	GASERANA GOLOLCHA	07-20-00-N	40-29-00-E	2040
3	32378	Amhara	SEMEN GONDAR	GONDAR ZURIA	12-32-00-N	37-12-00-E	1950
4	32499	Oromiya	MIRAB SHEWA	WALISONA GORO			2020
5	32554	SNNP	HADIYA	SORO			2020
6	32592	Amhara	SEMEN SHEWA	LAY BETNA TACH BET	10-03-00-N	35-55-00-E	1989
7	32730	Oromiya	JIMMA	SOKORU	07-52-00-N	37-25-00-E	2020
8	32733	Amhara	DEBUB WELLO	KALU	11-06-00-N	39-46-00-E	1900
9	202282	Oromiya	MISRAK WELLEGA	GUTO WAYU			1900
10	226040	Amhara	SEMEN GONDAR	DEMBIA	12-32-00-N	32-12-00-E	1945
11	226041	Amhara	B. DAR SPECIAL	BAHIR DAR	11-32-00-N	37-26-00-E	2010
12	230048	Oromiya	BALE	NENSEBO	06-64-00-N	39-01-00-E	1940
13	231214	Somali	JIGJIGA	JIGJIGA	09-32-00-N	42-33-00-E	2050
14	231263	Oromiya	ARSSI	JEJU	08-37-00-N	39-40-00-E	2030
15	240706	Oromiya	ARSSI	MERTI			1960
16	32103	Oromiya	MIRAB HARERGE	CHIRO	09-03-16-N	40-54-31-E	2000
17	241550	Amhara	MISRAK GOJAM	GUZAMN	10-14-39-N	37-45-15-E	2000
18	243842	Tigray	Central	KOLA TEMBEN	13-37-00-N	39-00-00-E	1990
19	243843	Tigray	Central	ADWA	14-10-00-N	38-51-00-E	2075
20	243848	Amhara	DEBUB GONDAR	KEMEKEM	12-07-00-N	37-49-00-E	2070

Table 2: Mean germination rate of field pea accessions subjected to different concentrations of NaCl in the laboratory experiment

S.No	Accessions	NaCl concentration (mM)			
		0	50	75	100
1	32499	5.54	7.50	9.75	12.00
2	243848	5.45	10.75	12.67	13.67
3	32052	5.27	7.70	8.55	10.50
4	32001	5.19	7.50	8.30	10.22
5	241550	5.00	10.25	11.75	13.33
6	32378	4.73	6.31	7.13	8.22
7	32730	4.61	9.08	13.50	14.00
8	32103	4.50	5.14	5.71	6.48
9	226040	4.10	4.81	6.29	6.67

10	32554	4.07	5.35	7.75	9.58
11	32592	4.00	4.63	5.40	7.77
12	32733	3.86	5.33	6.87	7.60
13	240706	3.80	4.92	5.09	5.69
14	202282	3.70	4.31	4.85	6.48
15	243842	3.56	3.99	6.02	7.33
16	231214	3.52	3.96	4.81	5.23
17	226041	3.50	6.45	10.00	11.83
18	231263	3.32	6.25	7.33	8.30
19	230048	3.22	4.26	5.22	6.14
20	243843	3.10	6.87	8.80	9.90
LSD ($\leq$ 0.05) 0.65					
CV(%) 5.93					

Field pea accessions responded differently to different NaCl concentrations and the mean value of germination rate varied from 3.10-5.54 in controls, 3.96-10.75 in 50mM treatment and 4.81-13.50 in 75mM and 5.23-14.00 in 100mM salinity level. This showed that every salt treatment had delayed germination as compared to the control and the accessions showed variations among themselves in response to salt stress.

The delay was more pronounced with higher salt concentrations (table 2). The results of our study are in agreement with Kaya *et al*.[18] who observed an increase in germination rates with the increasing NaCl stress.

According to Karagiizel[19], germination time in different plant species considerably increases with an increase in salt concentration. Salinity influenced germination time more dramatically than the germination percentage [20]. This means that increase in salt concentration results in prolongation of germination time. However, salinity caused different germination time delay amongst the accessions studied. Delayed germination has also been reported on pepper. Germination under salt stress can be used as a criterion for salt tolerance. Furthermore, the rapid germination may contribute to salt tolerance to some extent. Salinity stress negatively affects seed germination, either osmotically through reduced water absorption or ionically through the accumulation of Na^{+}andCl^{-}, causing an imbalance in the nutrient uptake and toxicity effect. Screening for salinity tolerance at germination and seedling stage has many advantages such as being less laborious, quick, and inexpensive as compared to investigations at mature growth stages [21].

Effect of salinity on germination percentageof field pea accessions

Salt stress significantly reduced germination percentage. On the basis of this important indicator of salt stress, high percent germination was observed in some studied accessions in the present study which indicates their potential to resist the salt stress. On the other hand, poor germination percentage was achieved in the other accessions studied this may be due to high accumulation of toxic salts i.e. Na^{+} and Cl^{-}, which may lower their water absorption potential, change certain enzymatic or hormonal activities inside the seed and decrease water up take through osmotic effect[22].

The result revealed that the germination of field pea accessions was adversely affected by all salinity treatments (table 3). That indicated the percent germination generally decreased with increasing salt concentration and the degree of reduction varied with the salinity levels and field pea accessions. At 50mM salinity level, accessions 231214 (93.33) followed by 230048 (86.67) exhibited high percent of germination while, accessions 240706 (83.33) and 226040 (76.67) had moderate germination percentage. At 75mM salinity level, accessions 231214 followed by 230048 were attained maximum germination percentage with values of 83.33 and 76.67 respectively. Similarly, accessions 243842, 32103, 226040, 32592, 202282 and 240706 showed good values ranging from 63.33-73.33. While, the remaining twelve accessions were varied in range between 33.33-56.67. At the highest salt concentration (100mM), the germination ranged from 30-76.67%. Accession 231214 gave high values of germination percentage compared to the other accessions while, accessions 241550, 32001, 243848 and 32730 had relatively low germination percentages compared to the other accessions.More pronounced reduction of germination percentage was observed at higher salinity level (100mM) of salt concentrations as compared to control. The present result is in line with findings indicating that salinity stress severely reduces germination percentage in safflower[23].

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Table 3: Mean germination percentage of field pea accessions subjected to different concentrations of NaCl in the laboratory experiment

S.No	Accessions	NaCl concentration (mM)			
		0	**50**	**75**	**100**
1	240706	100.00	83.33	73.33	66.67
2	32592	100.00	73.33	66.67	56.67
3	243843	100.00	56.67	46.67	43.33
4	32554	100.00	46.67	36.67	33.33
5	241550	100.00	40.00	36.67	30.00
6	231214	96.67	93.33	83.33	76.67
7	32052	96.67	53.33	43.33	36.67
8	226040	93.33	76.67	66.67	63.33
9	32733	93.33	66.67	56.67	50.00
10	231263	93.33	43.33	40.00	33.33
11	243848	93.33	40.00	33.33	30.00
12	230048	90.00	86.67	76.67	66.67
13	243842	90.00	70.00	63.33	60.00
14	32378	90.00	63.33	53.33	36.67
15	32103	86.67	70.00	66.67	63.33
16	32499	86.67	53.33	43.33	33.33
17	32730	86.67	36.67	33.33	30.00
18	202282	83.33	80.00	70.00	63.33
19	32001	83.33	46.67	43.33	30.00
20	226041	80.00	46.67	40.00	33.33
		LSD ($\leq$ 0.05)	7.08		
		CV(%)	7.87		

Effect of salinity on seedling shoot length of field pea accessions

All accessions responded differently to salt stress with respect to mean seedling shoot length. The mean of seedling shoot length ranging from 10.29- 17.66cm in the control, 6.52-16.35cm in 50mM, 5.45-13.87cm in 75mM and 4.40-12.96cm in 100mM concentration of NaCl (table 4). Increment of salinity level had significantly reduced mean seedling shoot length in all accessions. Nevertheless, the reduction was more pronounced at higher salinity levels. At 50mM NaCl concentration, accession 226040 produced relatively high seedling shoot lengths with 95.75% compared to respective control whereas, accession 243848 produced relatively low values of 57.6%. At 75 mMNaCl concentration, accession 226040 produced relatively high values of seedlings shoot length with 86.13% compared to the control whereas, accessions 243848 and 32730 had relatively attained low values of seedling shoot length with 48.14% and 49.85% respectively compared to control. Similarly, at 100mM NaCl concentration, accessions 32592, 32103 and 243842 produced relatively higher values of seedling shoot length with 78.66%, 78.32% and 78.05% respectively compared to the control while, accession 32730 produced the lowest values of seedling shoot length with 40.89% compared to other accessions. The finding of this study indicated that, as salinity level increased from 50mM to 100mM, the mean seedling shoot length was reduced highly in all field pea accessions studied. Similar outcome were reported by Khajeh-Hosseini*et al.*, [24], El-Hendawy*et al.,*[25]in soybean and wheat respectively.

Table 4: Mean seedling shoot length of field pea accessions treated with different concentrations of NaCl in the laboratory experiment

S.No	Accessions	Salt Concentrations (mM)			
		0	50	75	100
1	231214	17.66	16.35	13.87	12.96
2	230048	16.74	15.31	13.33	12.68
3	240706	16.67	14.69	13.46	12.48
4	32592	16.26	14.71	13.47	12.79
5	32733	16.23	13.45	12.04	9.87
6	226040	16.01	15.33	13.79	12.01

7	202282	15.81	14.15	12.00	11.62
8	243843	15.32	11.55	10.88	9.37
9	32103	15.22	13.84	12.29	11.92
10	243842	15.17	14.11	12.20	11.84
11	32378	14.49	10.24	8.30	7.62
12	32499	14.22	11.07	8.26	7.45
13	32554	14.11	9.50	7.98	7.59
14	32052	13.43	10.27	8.51	7.76
15	241550	13.41	8.82	7.89	7.00
16	32730	13.28	8.12	6.62	5.43
17	226041	13.06	9.06	7.29	5.95
18	32001	12.79	8.42	6.85	5.69
19	243848	11.32	6.52	5.45	4.82
20	231263	10.29	7.42	5.46	4.40
LSD($\leq$ 0.05)		0.52			
CV (%)		2.93			

Even at the lowest salt concentration, seedling shoot length was significantly reduced. The significant reduction in seedling shoot length may be attributed to the toxic effect of sodium chloride and unbalanced nutrient uptake by seedlings. These deleterious effects of salinity may result in a significant decrease in photosynthesis and increase in transpiration rate leading to a shortage of assimilate to the developing organs, thus slowing down growth or stopping it entirely[26]. As salinity enhances osmotic pressure leading to reduction in water absorbance, cell division and differentiation are inhibited, which adversely affects metabolic and physiological processes. This causes more delay in initiation of germination followed by prolonged seed germination duration (Kang and Saltvett, [27] and ultimately reducing shoot and rootlength [28].

Effect of salinity on seedling root length of field pea accessions

The mean seedling root length ranging from 6.99-10.07cm in the control, 1.83-6.41cm in 50mM, 1.20-5.19cm in 75mM and 0.95-4.43cm in 100mM concentrations(table 5). The increment in salinity level had significantly reduced the mean seedling root length of all accessions however; the reduction in seedling root length was not same in all the accessions at different concentration of NaCl. Accessions, 32554 and 231214 were observed to have relatively higher root lengths at the control whereas, accession 243848 had the lowest value for seedling root length. At 50mM NaCl concentration, accessions 230048 and 32592 achieved higher reduction of 23.13% and 23.40% respectively compared to the control while, accessions 240706 and 32103 had the intermediate values of 27.49% and 28.72% reduction seedling root lengths compared to the other accessions respectively. Moreover, accession 226041 was observed to have the lowest seedling root length with 76.23% reduction compared to the control. Similarly, at 75mM salt concentration accession 230048 produced the highest value for seedling root length with 58.59% compared to the control while accession 241550 had relatively low values for seedling root length with 16.34% production with respect to the other accessions. Furthermore, at the highest salinity level, 100mM salt concentrations accession 230048 observed to have the longest seedling root length with 51.62% reduction compared to control whereas, accession 241550 observed to have the shortest value for seedling root length with 89.24% reduction with respect to the other accessions

Table 5: Mean seedling root length of field pea accessions subjected to different concentrations of NaCl in the laboratory experiment

S.No	Accessions	NaCl concentration (mM)			
		0	50	75	100
1	32554	10.07	4.52	3.31	1.92
2	231214	10.02	6.41	5.19	4.43
3	241550	9.30	2.92	1.52	1.00
4	226041	9.17	2.81	2.13	1.14
5	226040	9.14	5.55	4.17	3.66

6	32001	8.49	4.16	2.71	1.43
7	202282	8.29	5.36	3.94	3.34
8	32052	8.15	3.82	2.51	2.27
9	32592	8.12	6.22	4.30	3.55
10	32733	8.09	4.38	2.99	2.78
11	230048	8.04	6.18	4.71	3.89
12	231263	7.96	3.10	1.92	1.47
13	240706	7.93	5.75	4.21	3.60
14	243848	7.77	1.83	1.20	0.95
15	32499	7.47	2.91	1.68	1.26
16	32378	7.38	3.18	2.08	1.69
17	243842	7.37	4.87	3.48	3.09
18	32103	7.24	5.16	3.70	3.22
19	243843	7.07	3.60	2.38	2.10
20	32730	6.99	2.46	1.36	1.03
LSD($\leq$ 0.05) 0.12					
CV(%) 1.78					

The result in the mean seedling root length revealed that there was a great difference among field pea accessions in response to salt stress. This may be due to genetic variation among the field pea accessions. In line with this result, Jamil*et al*. [4] and Rahman*et al.*[29]on vegetables species and wheat reported maximum reduction in seedling root length as salinity level increased. The seedling root length was generally decreased with increasing salinity level and degree of reduction varied with salinity levels and among the accessions.

The present investigation confirmed that better seedling root length was observed at lower salinity concentration (50mM) and at the control conditions and great reduction on seedling root length was observed at higher salt concentration (100mM). The profound effect of salinity on the length of shoot and root is because a high salt concentration in the external medium creates high osmotic potential reducing the availability of soil water to growing cells. The reduction in moisture availability may affect cell elongation thereby affecting cell expansion and cell protrusion of embryo axis which may hamper growth of root and shoot. Since, hydration of seeds is an essential prerequisite for synthesis and activation of various enzymes, the reduction in water uptake also has profound effect on metabolic machinery of the seedlings [22].

Effect of salinity on seedling shoot length to seedling root length ratioof field pea accessions

In the laboratory experiment accession243843 gave significantly higher mean seedling shoot to root ratios in the control whereas, accession 231263 showed the lowest value of seedling shoot to root ratio (table 6).Accession 32499 had significantly higher mean seedling shoot to root ratios in the 50mM salinity level with 3.81 while, accession 32001 was found to have significantly lower values than the other accession with 2.02.In the 75mM NaCl salinity level, accession 241550 achieved the highest seedling shoot to root ratio with 5.17whereas, accession 32554 had significantly lower values of seedling shoot to root ratio with 2.41 compared to other accessions. In the 100mM NaCl salinity level, accession 241550 attained significantly higher mean seedling shoot to root ratio with 7.00 whereas accession 231214 had the lower values of seedling shoot root ratio with 2.92 compared to other accessions.

Table 6: Mean seedling shoot length to seedling root length ratio of field pea accessions subjected to different concentrations of NaCl in the laboratory experiment

S.No	Accessions	NaClConcentrations(mM)			
		0	50	75	100
1	243843	2.17	3.21	4.57	4.47
2	32103	2.10	2.68	3.33	3.70

3	240706	2.10	2.55	3.20	3.47
4	230048	2.08	2.48	2.83	3.26
5	243842	2.06	2.90	3.51	3.83
6	32733	2.01	3.07	4.02	3.55
7	32592	2.00	2.36	3.14	3.60
8	32378	1.96	3.22	4.00	4.49
9	202282	1.91	2.64	3.05	3.48
10	32730	1.90	3.30	4.87	5.26
11	32499	1.90	3.81	4.92	5.91
12	231214	1.76	2.55	2.67	2.92
13	226040	1.75	2.76	3.31	3.28
14	32052	1.65	2.69	3.39	3.42
15	32001	1.51	2.02	2.52	4.00
16	243848	1.46	3.58	4.55	5.10
17	241550	1.44	3.01	5.17	7.00
18	226041	1.42	3.22	3.42	5.22
19	32554	1.40	2.10	2.41	3.97
20	231263	1.29	2.39	2.85	3.00
LSD($\leq$ 0.05)		0.28			
CV (%)		5.63			

The result indicates that regarding seedling shoot-root length ratios in the laboratory experiment most accessions showed increment of seedling shoot to root ratio as the salinity level increased in the growth media. The results in this study are in agreement with Abdelhamid*et al*.[30] who reported significant differences in seedling shoot-root length due to salinity among fababean genotypes. This result was also in agreement with the results of Geressu and Gezahagn,[31], who reported significant difference for salinity treatments and treatment interactions for seedling shoot to root ratio of sorghum genotypes.

Effect of salinity on seedling shoot fresh weight of field pea accessions

In the laboratory experiment mean seedling shoot fresh weight ranged from 0.54-1.14 in control, 0.09-0.87 in 50mM, 0.05-0.60 in 75mM and 0.03-0.43 in 100mM salt concentrations (table 7). At 50mM NaCl concentration accession 231214 had higher seedling shoot fresh weight values with 0.87gram compared to the control whereas, accession 241550 had lower values of seedling shoot fresh weight with 0.09gram. At 75mM salt concentration, accession 231214 and 226040 produced relatively high values of seedling shoot fresh weight with 52.63% and 52.53% compared to the control respectively while, accession 241550 recorded relatively low values of seedling shoot fresh weight compared to the control with 94.25% reduction and considered as salt sensitive. Likewise, at 100mM NaCl salinity level accession 226040 achieved the highest value of seedling shoot fresh weight with 60.60% reduction compared to control whereas, accession 243842 had intermediate values of seedling shoot fresh weight with 61.29% reduction compared to control while, accession 241550 achieved low values of seedling shoot fresh weight with 96.55% reduction compared to the other accessions. This result exhibited that seedling shoot fresh weight decreased with increasing salinity levels however, the reduction varied with type of accessions and salinity levels. This result is consistent with the findings ofJamil*et al*., [4] and Afzal*et al*.,[32] in cabbage and sugar beet respectively.

Table 7: Mean seedling shoot fresh weight of field pea accessions subjected to different concentrations of NaCl in the laboratory experiment

S.No	Accessions	NaCl Concentrations (µM)			
		0	50	75	100
1	231214	1.14	0.87	0.60	0.43
2	32052	1.13	0.43	0.25	0.15
3	243848	1.08	0.41	0.30	0.14
4	243843	1.07	0.33	0.19	0.13
5	226040	0.99	0.70	0.52	0.39
6	32378	0.97	0.34	0.18	0.13
7	231263	0.96	0.12	0.08	0.05
8	32733	0.94	0.40	0.28	0.17

9	243842	0.93	0.58	0.43	0.36
10	202282	0.88	0.53	0.45	0.36
11	241550	0.87	0.09	0.05	0.03
12	32554	0.83	0.24	0.14	0.10
13	240706	0.82	0.55	0.40	0.28
14	230048	0.80	0.71	0.43	0.32
15	32592	0.76	0.44	0.34	0.21
16	32103	0.75	0.52	0.37	0.26
17	32499	0.73	0.18	0.12	0.05
18	32001	0.69	0.16	0.10	0.06
19	32730	0.67	0.18	0.12	0.07
20	226041	0.54	0.13	0.07	0.03
LSD ($\leq$ 0.05) 0.04					
CV(%) 6.35					

This is explained by the fact that high salt concentration decreases the water potential of the growth medium which leads to the reduction in cell turgor. This low cell turgor inhibits the cell elongation and cell division so that plant growth slows. Some accessions like 230048 and 202282 performed well at higher NaCl salinity levels with regard to shoot fresh weight and this might have been due to their successful maintenance of cell turgor under saline conditions while, non-tolerant accession like 241550 could not maintain the proper cell turgor.

Toxic ions like Na^+ and Cl^- also limits cell elongation and cell differentiation which may lead to the reduction in plant biomass, root and shoot lengths in salinized plants of tested pea accessions. Under saline conditions, fresh and dry matter accumulation is the ultimate goal to enhance the plant productivity in pea. The reduction may be due to many reasons such as lack of maintenance of turgor, sodium/chloride ion toxicity and disturbances in metabolic path ways. Fresh and dry weight reduction under saline conditions may be due to the reduction in above ground biomass allocation.

Effect of salinity on seedling root fresh weight of field pea accessions

The NaCl treatments used at laboratory experiment caused significant reduction in seedling fresh root weight. Although there was higher seedling fresh root weight in control however, the mean seedling fresh root weight reduces as salinity level increased. In the laboratory experiment, the mean seedling root fresh weight ranged between 0.50-1.05gram in control, 0.06-0.73gram in 50mM, 0.03-0.51gram in75mM and 0.03-0.37gram in 100mM (table 8). At control, accession 231214 had the highest value of seedling root fresh weight. Similarly, at 50mM NaCl concentration accession 230048 achieved the highest mean of seedling root fresh weight with 16.39% reduction compared to the control while accession 226041 had the lowest value with 88.46% reduction. At 75mM salinity level, accession 230048 performed well compared to other accessions with value 0.41gram whereas, accessions 226040 and 240706 performed moderately compared with the rest of the accessions. Accession 226041 had the lowest value for seedling root fresh weight with 0.03gram. Furthermore, at 100mM salinity level, accession 230048 had relatively higher values with 0.30gram compared to the control while, accession 226041 had lower values of 0.03gram compared to the other accessions.

Table 8: Mean seedling root fresh weight of field pea accessions subjected to different concentrations of NaCl in the laboratory experiment

S.No	Accessions	NaCl concentration (mM)			
		0	50	75	100
1	231214	1.05	0.73	0.51	0.37
2	243848	0.73	0.31	0.22	0.12
3	241550	0.72	0.18	0.13	0.10
4	32733	0.70	0.27	0.19	0.14
5	32052	0.67	0.18	0.13	0.10
6	243842	0.64	0.35	0.23	0.17
7	226040	0.63	0.47	0.38	0.26
8	230048	0.61	0.51	0.41	0.30

9	243843	0.60	0.22	0.14	0.10
10	32103	0.59	0.36	0.25	0.16
11	32499	0.59	0.14	0.08	0.05
12	32001	0.58	0.11	0.08	0.07
13	32554	0.57	0.13	0.10	0.07
14	240706	0.57	0.43	0.34	0.22
15	202282	0.56	0.34	0.24	0.15
16	32592	0.53	0.34	0.26	0.21
17	226041	0.52	0.06	0.03	0.03
18	32730	0.51	0.11	0.08	0.05
19	231263	0.51	0.10	0.07	0.04
20	32378	0.50	0.09	0.06	0.04
LSD ($\leq$ 0.05) 0.05					
CV(%) 10.68					

The present investigation indicated that as salinity level increased the seedling root fresh weight decreased however, the reduction varied between salinity levels and type of accessions studied. This result is in line with previous findings of pea who confirmed that seedling growth was decreased as salinity level increased. Ok cu*et al*. [33] indicated that decrease in seedling fresh and dry weights was due to the restricted provider of metabolites to younger growing organs, since metabolic performance was considerably disturbed at higher levels of salinity and also due to the low water absorption and toxicity of NaCl. Heshmat*et al*. [34] in cowpea and Armin *et al*.[35] in watermelon reported that seedling growth was ceased by NaCl. The fresh and dry weight traits were also considered key indicator of seedling vigor. Both fresh as well as dry weight of root and shoot were affected greatly with higher NaCl stresses. The higher the NaCl concentrations, the lesser the root and shoot weight. It could be caused by reduced water potential leading to imbalanced water and nutrient supply due to NaCl toxicity that hindered dry matter accumulation. Similar results were also recorded in Pak Choi type of *Brassica rapa*, where root fresh weight was more affected by salinity at 50 MmNaCl [4].

Effect of salinity on seedling shoot dry weight of field pea accessions

The decline in seedling shoot dry weight was more pronounced at higher NaCl salinity levels as compared to the control. The mean seedling shoot dry weight in laboratory experiment ranged from 0.21-0.42gram in the control, 0.04-0.35gram in 50mM, 0.02-0.22gram in 75mM and 0.01-0.15gram in 100mM (table 9).The present study accession 231214 exhibited the highest seedling shoot dry weight with 0.25gram at 50mM salt concentration compared to control whereas, accessions 226041 attained the lowest mean with 0.04gram of seedling shoot dry weight compared to control. Similarly, at 75mM NaCl salt concentration accessions 243842 performed well with 34.78% reduction compared to the control. In contrast to this, accessions 241550 and 226041 had relatively lower mean values for seedling shoot dry weight with 91.30% and 90.48% reduction respectively compared to control and these are considered as salt sensitive. Furthermore, at 100mM NaCl salt concentration accession 32103 attained the highest seedling shoot dry weight with 50% reduction with respect to the other accessions studied while accession 32730 had relatively low values of seedling shoot dry weight with 96% reduction.

Table 9: Mean seedling shoot dry weight of field pea accessions subjected to different concentrations of NaCl in the laboratory experiment

S.No.	Accessions	NaCl concentration (mM)			
		0	50	75	100
1	32001	0.42	0.08	0.05	0.03
2	202282	0.41	0.35	0.22	0.15
3	240706	0.39	0.25	0.17	0.12
4	32592	0.38	0.18	0.13	0.10
5	32554	0.32	0.10	0.08	0.05
6	243843	0.32	0.09	0.05	0.03
7	226040	0.30	0.22	0.17	0.13
8	32378	0.30	0.13	0.09	0.06
9	243848	0.30	0.11	0.07	0.05
10	32499	0.30	0.11	0.06	0.02

11	32052	0.30	0.13	0.09	0.06
12	32733	0.29	0.13	0.08	0.05
13	231214	0.27	0.25	0.15	0.12
14	230048	0.27	0.20	0.13	0.08
15	32730	0.25	0.07	0.04	0.01
16	231263	0.24	0.07	0.04	0.02
17	243842	0.23	0.20	0.15	0.11
18	241550	0.23	0.05	0.02	0.01
19	32103	0.22	0.19	0.14	0.11
20	226041	0.21	0.04	0.02	0.02
LSD ($\leq$ 0.05) 0.02					
CV(%) 8.32					

This result elucidates that salinity antagonistically caused reduction in seedling shoot dry weight. The increment of salinity caused significant reduction in seedling shoot dry weight and this result agree with Karimi*et al*.[36] who claimed that increased salinity levels caused remarkable decrease in shoot dry weight. The present study is also in line with earlier studies in safflower by Ghazizade*et al*.[37] who reported that adding NaCl resulted in decrease of the shoot dry weight and Mensuh*et al*.[38]who reported the increment of salinity cause progressively reduction on shoot dry weight in groundnut. This may be a result of a combination of osmotic and specific ion effect of Cl^- and Na^+[39]. The reduction in shoot dry weight could also be associated with reduced rate of leaf production, hence low number of leaves leading to reduced photosynthesis and accumulation of dry matter. Root injury and death due to ionic toxicity may have affected water uptake by the plants and as a result increased water deficit in the plants leading to decreased net photosynthesis, which in turn may have affected shoot growth. Water deficit may as well occur as the result of lowered water potential of the soil solution and restricting root water uptake. Reduction in dry weight of plant tissues reflects the increased metabolic energy cost and reduced carbon gain, which are associated with salt adaptation [40]. It also reflects salt impact on tissues, reduction in photosynthetic rates per unit of leaf area and attainment of maximum salt concentration tolerated by the fully expanded leaves [3]. At high-salt concentration, the plants could not regulate ion concentration, since there may have been severe physiological dysfunctions leading to decreased growth rates and eventually cell death leading to death of whole plant. Shoot and root damages caused by ion toxicity, osmotic effects or both may have contributed to the observed sharp drop in dry weights preceding the death of highly-stressed plants.

Effect of salinity on seedling root dry weight of field pea accessions

The mean seedling dry weight of root ranged from 0.14- 0.28gram, 0.02-0.25gram, 0.01-0.17gram and 0.01-0.14gram in the control, 50mM, 75mM and 100mM concentrations of NaCl, respectively (table 10). The results of this study showed that the seedling root dry weight was affected by salinity and their effect varied depending on salinity level and accessions.

Table 10: Mean seedling root dry weight of field pea accession subjected to different concentrations of NaCl in the laboratory experiment

S.No.	Accessions	NaCl concentration (mM)			
		0	50	75	100
1	202282	0.28	0.25	0.17	0.14
2	243843	0.24	0.09	0.04	0.02
3	226040	0.23	0.19	0.14	0.13
4	32052	0.23	0.06	0.05	0.04
5	32001	0.23	0.03	0.02	0.01
6	231214	0.21	0.19	0.13	0.11
7	230048	0.20	0.17	0.10	0.09
8	32103	0.20	0.15	0.13	0.10
9	32733	0.20	0.11	0.05	0.04
10	231263	0.20	0.07	0.03	0.01
11	243842	0.19	0.17	0.13	0.11
12	32499	0.19	0.08	0.06	0.02
13	240706	0.17	0.14	0.12	0.09

14	32592	0.17	0.14	0.10	0.07
15	32554	0.17	0.04	0.03	0.02
16	241550	0.15	0.09	0.07	0.04
17	226041	0.15	0.02	0.01	0.01
18	32378	0.14	0.04	0.03	0.02
19	32730	0.14	0.03	0.02	0.01
20	243848	0.14	0.02	0.01	0.01
LSD ($\leq$ 0.05) 0.02					
CV(%) 9.76					

At 50mM salinity level, accession 231214 produced relatively higher seedling root dry weight which is 90.48% of the value it had compared to control whereas, accessions 243842 and 202282 produced intermediate values of seedling root dry weight with 89.47% and 89.29% compared to control respectively. In contrast, accession 226041 produced lower values with 13.33% compared to control. Accessions 32001 and 226041 had relatively lower values of seedling root dry weight with 86.96% and 86.67% reduction compared to control these could be described as salt sensitive. At 75mM salinity level, accession 240706 produced the highest value 70.59% seedling root dry weight compared to control. On the other hand, accessions 243848, 226041 and 32001 had lower values with 92.86%, 93.33% and 91.30% reduction respectively. At 100mM salinity level, accession 243842 had relatively high values of 0.11gram compared to control whereas, accessions 32001 and 231263 had lowest performance with similar values of seedling root dry weight with 0.01gm. The mean seedling root dry weight decreased as salinity level increased. However, the reduction varied among the accessions and salinity levels studied. The present investigation is in agreement with previous reports by Asfawkinfe michael[41] in haricot bean and Akram*et al*. [42] in sunflower which indicated that salinity stress decreased the mean seedling root dry weight considerably. Reduction in root and seedling growth under saline conditions may either be due to decrease in the availability of water or increase in sodium chloride toxicity. Growth inhibition by salt stress also occurs due to the diversion of energy from growth to maintenance. For maintenance under salt stress plants need to regulate ion concentration in various organs and within the cells by synthesis of organic solutes for osmo regulation or protection of macro molecules and for maintenance of membranes integrity[43].The reduction in dry weight of plumule and radicle (seedling) as result of increased salinity is a normal phenomenon and probably the result of low water absorbance by germinating seeds. Also, Amiri*et al*. [44] reported that the effect of salinity stress on germination and seedling growth indices of *Cynarascoolymus* and *Echinacea purpurea* reported that seedling dry weight was decreased by increasing salinity stress in both medicinal plants.

Ranking accessions for salt tolerance

Selecting the best performed field pea accessions from the laboratory experiment. Score was calculated based on the number of germinated seeds and the average seedling shoot length. Based on this result accession 231214 ranked first with 227.28 while accession 32730 ranked the last with values 51.08 (table 11). Generally eight accessions were obtained from laboratory experiment, which was best performed accessions 231214, 240706, 230048, 32103, 202282, 32592, 226040 and 243842 were selected for pot experiment since they performed relatively well in all aspects. These results can be related to some earlier studies in which lines identified as salt tolerant at the earlier growth stage showed tolerance when tested at the later growth stage reported by Fawzi *et al.* [45] on study of some legumes and forage cultivars.

Table 11: Ranking of the field pea accessions subjected to different concentrations of NaCl under in the laboratory experiment

S.No	Accessions	Score	Rank
1	231214	227.28	1
2	240706	196.43	2
3	230048	184.44	3
4	32103	161.15	4
5	202282	158.86	5
6	32592	146.38	6
7	226040	143.00	7
8	243842	121.75	8
9	32733	113.78	9

10	243843	93.66	10
11	32052	93.41	11
12	32554	83.45	12
13	32378	79.52	13
14	32499	64.37	14
15	231263	64.22	15
16	32001	57.32	16
17	241550	56.42	17
18	226041	53.91	18
19	243848	51.15	19
20	32730	51.08	20

Table: 12 Pearson's correlation coefficients for plant growth, morphological and biomass traits under laboratory experiment

	GP	GR	SSL	SRL	SSRR	SSFW	SRFW	SSDW	SRDW
GP	1.00	-0.86**	0.89**	0.92**	-0.75**	0.92**	0.89**	0.88**	0.88**
GR		1.00	-0.82**	-0.82**	0.82**	-0.75**	-0.71**	-0.76**	-0.79**
SSL			1.00	0.83**	-0.61**	0.93**	0.92**	0.90**	0.88**
SRL				1.00	-0.84**	0.91**	0.92**	0.89**	0.86**
SSRR					1.00	-0.73**	-0.70**	-0.73**	-0.67**
SSFW						1.00	0.94**	0.89**	0.87**
SRFW							1.00	0.85**	0.84**
SSDW								1.00	0.90**
SRDW									1.00

Where ** = highly significant at p< 0.01, GP = germination percentage GR= germination rate, SSL = seedling shoot length, SRL = seedling root length, SSRR = Shoot to root ratio, SSFW = shoot fresh weight, SRFW = root fresh weight, SSDW = shoot dry weight and SRDW = root dry weight

Correlation Analysis

Pearson's correlation coefficients (r) that were computed for nine traits in the laboratory experiment. The correlation coefficient among most of the quantitative characters was highly significant (p< 0.01). In the laboratory screening experiment, germination percentage showed positive and highly significant (p<0.01) correlation with seedling shoot length, seedling root length, seedling shoot fresh weight, seedling root fresh weight, seedling shoot dry weight and seedling root dry weight. The correlation between germination rate and germination percentage was highly significant and negatively correlated (r = -0.86**, p<0.01), showing that germination rate was delayed while germination percentage was decreased. Germination rate also showed a negative and highly significant correlation with almost all traits except seedling shoot to root ratio. In addition seedling shoot to root ratio also showed positively and highly significant correlation with germination rate (r = 0.82**, p<0.01) whereas, negatively correlated with almost all traits.

CONCLUSION

The result of the petri dish experiment showed that all the traits considered showed highly significant variation among accessions, treatments and accessions with treatments interactions. Every salt treatment delayed the emergence of plumule and radicle compared to the control and the delay was more pronounced at higher salt concentrations. The mean value indicated that the maximum germination percentage was observed on accession 231214 in all treatments whereas; the minimum germination percentage was obtained on accessions 32730 and 243848. Seedling shoot length and seedling root length was significantly affected by

NaCl salt concentration. The lowest mean seedling root length was attained by accessions 241550 and 226041. The highest seedling root length attained by accession 230048. The highest mean seedling shoot length was recorded for accessions 226040 in all salt treatments whereas, accession 243848 achieved the lowest mean seedling shoot length. The increment of seedling shoot to root ratio in response to increased salt concentrations indicated that the mean seedling root length was more salt–affected than the mean of seedling shoot length at higher salinity levels. It was also noted that seedling shoot fresh weight, seedling root fresh weight, seedling shoot dry weight and seedling root dry weight were highly significantly influenced by NaCl stress in the laboratory experiment. Generally, the seedling shoot fresh weight, seedling root fresh weight, seedling shoot dry weight and seedling root dry weight of the accessions 231214, 230048, 226040, 32592, 240706, 243842, 32103 and 202282 were the highest compared to other accessions.

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CONFLICT OF INTEREST

There is no conflict of interest.

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