

## International Journal of Current Trends in Pharmacobiology and Medical Sciences

Volume 2 • Number 1 (January-2017) • ISSN: 2456-2432

Journal homepage: www.ijctpms.com



**Original Research Article** 

# Adaptability of Dekoko (*Pisum sativum* var. *abyssinicum*) Seedlings to Salinity Stress in *In Vitro* Culture Conditions

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#### Abstract

Dekoko (Pisum sativum var. abyssinicum) is one of the most important food legumes grown in south Tigray and north Wollo, northern Ethiopia. It is one among the most important food legumes in terms of protein content. It grows mixed and alone with many cereal crops growing in north Ethiopia. This study was conducted in 2015/2016with the objective of selecting adaptable and relatively high yielding P. sativum var. abyssinicum accessions under different salt (NaCl) concentrations at laboratory conditions. The seeds for the 30 accessions were obtained from nine districts of two regional states of north Ethiopia with different attitudinal ranges 700 m.a.s.l being the lowest and 3148 m.a.s.l the highest. Of the 30 on farm and wire house tested accession, six better performing local accessions, three from Ofla (T-001/08OF, T-002/08OF and, T-003/08OF), one from Sirinka (TA-026/15Sr), one from Emba-Alaje (T-025/15E/A), and one from Endamohoni (T-023/08MW) were selected for this study for salt stress resistances in controlled condition by priming in four salt treatment levels (5 dS/m, 7 dS/m, 9 dS/m and 15 dS/m). Distilled water (0 dS/m) was used as control. Fifty (50) surface sterilized seeds per Petri dish were sown for the four salt treatments and the control. Accessions T-001/08 from Ofla and T-023/08 from Endamohoni showed good growth performance at 5 dS/m. However, TA-026/15Sr from Sirinka and T-025/15E/A from Betmara (Emba-Alaje) respond positively up to 7dS/m. At higher salinity level growth performance decreased with increasing salinity stress. But, T-023/08MW, T-001/08OF,TA-026/15Sr followed by T-025/15E/A from lower to the higher resistances respectively could withstand lower to medium concentrations of salinity as compared to the other accessions.

#### Article Info

Accepted: 11 January 2016 Available Online: 25 January 2017

## Keywords

Endamohoni Growth performance Ofla Salinity stress

#### Introduction

It is approximated that the incessantly growing human population will increase global food production requirement by more than half by 2050 (Wild, 2003). Increasing food production through expansion of cultivation area will become challenging in the future due

to progressive soil degradation including soil salinization and insufficient farmlands (Wild, 2003). The adverse effects of salinity include not only low agricultural production and low income due to high production costs, but also due to soil erosion and ecological imbalance (Hu and Schmidhalter, 2002). Saline soil fertility can be improved by cultivating legume plants that increase

fertility due to their symbiotic capacity to enrich the soil with nitrogen (Keneni et al., 2013).

The demand for productivity and homogeneity in peas, as in other crops, has resulted in a limited number of highyielding varieties at the price of the loss of heterogeneous traditional local varieties (landraces) through genetic erosion (Smýkal et al., 2015). Conversely, Landraces preserve much of this diversity lost and comprise the genetic resources for breeding new crop varieties to help cope with environmental and demographic changes that enhance soil fertility (Esquinas-Alcazar, 2005; Smýkal et al., 2015). Grain legumes take up an exceptional position in world agriculture by virtue of their high protein content and capacity to fix atmospheric nitrogen. Field pea (Pisum sativum L.) is grown in many countries and currently ranks fourth among the pulses in the world with cultivated area of 6.33 million ha (FAO, 2012). Field pea is known to be grown in Ethiopia since antiquity (Keneni et al., 2013). Currently, the crop is the fourth most important pulse crop in Ethiopia, preceded only by faba bean, haricot bean and chickpea in terms of both area coverage and total national production (CSA, 2011; Berhane and Berhanu, 2016). There are two botanical cultivars of Pisum sativum cool season food legumes (CSFLs) known to grow in Ethiopia largely produced by subsistence farmers and serve as supplementary protein sources and soil fertility restorers, namely P. sativum var. sativum and P. sativum var. abyssinicum (Keneni et al., 2013; Berhane and Berhanu, 2016). Their yield are very low, mainly limited by soil fertility (Yemane and Skjelvåg, 2002; Berhane and Berhanu, 2016), because they are cultivated in poor soils, often without fertilization. P. sativum var. abyssinicum is capable of producing seed yield of up to 1.95 t/ha under phosphorus fertilization. It is known for its high market price (more than double of the price of faba bean and field pea) and for its food preference though resistances to salt stress is not known (Hadis and Dargie, 2013; Berhane and Berhanu, 2016).

Many crops exhibit a broad spectrum of response to salt stress but numerous have shared common responses that their growth and eventually yield reduced under salinity. Salt stress reduces dry matter content, increases the root to shoot ratio and reduces the leaf size, which all lead to yield reduction (Munns and Tester, 2008). Survival of the plant in unfavorable conditions requires improvement in the metabolism, including accumulation of protective compounds such as compatible solutes (e.g. proline) for osmotic adjustment, proteins and

antioxidants for oxidative stress mitigation (Ashraf and Foolad, 2007). Understanding of mechanisms that alleviate the impact of salt on plant growth and mechanisms of salinity tolerance at the whole-organism, organelle and molecular levels allows improving salt tolerance of crops both in irrigable and non-irrigable lands of the world under the changing climate scenario.

Although, disregarded by breeders, P. sativum var. abyssinicum is characterized by a high protein content in seeds, low soil fertility requirements and, unusual among legume plants, tolerant to different pH and soil type and especially to drought and moisture stress (Girmay et al., 2014; Berhane and Berhanu, 2016). These features make P. sativum var. abyssinicum plant selective and alternative to other high-protein legumes such as peas or beans, which require better growing conditions. Beyond the health value contribution it provides, P. sativum var. abyssinicum is a good nitrogen fixer and improves the yield of succeeding crops (Keneni et al., 2013). In the in vitro culture conditions, P. sativum var. abyssinicum is considered as less recalcitrant to regeneration of other similar plants contrasting to grass pea (Barpete et al., 2014; Tsegay and Gebreslassie, 2014). Even though P. sativum var. abyssinicum stands out next to grass pea with resistance to various abiotic stresses (Tsegay and Gebreslassie, 2014), mechanisms of adaptation to these stresses at the physiological and molecular level are assignments remain to be done (Berhane and Berhanu, 2016). There is little information on the effect of salinity on seed germination, growth and development of P. sativum var. abyssinicum seedlings and the whole plant (Tsegay and Gebreslassie, 2014). Therefore, the aim of this study was to identify possible adaptation growth responses of six on farm and wire house promising accessions of P. sativum var. abyssinicum to salinity stress. By doing so, it is possible to select adaptable and relatively high yielding accessions.

#### Materials and methods

This study was conducted in 2015/2016 at Bahir Dar University botany laboratory using saline growth media to validate and select the on farm as well as wire house agronomically better performers, adaptable and relatively high yielding *P. sativum* var. *abyssinicum* accessions under different salt (NaCl) concentrations at controlled condition. The seeds of 30 accessions (Table 1) were obtained from nine districts of two regional states of Ethiopia with different attitudinal ranges. Of the 30 on farm and wire house investigated

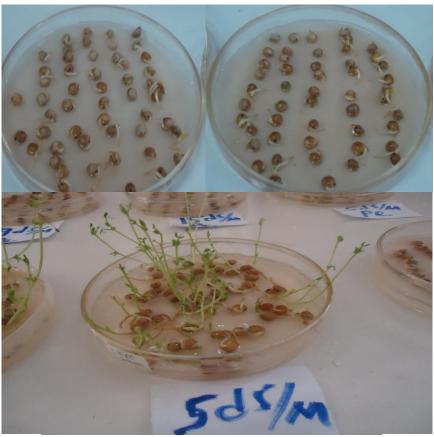
accession, six better performing local accessions, three Ofla (T-001/08OF, T-002/08OF 003/08OF), one from Sirinka (TA-026/15Sr), one from Emba-Alaje (T-025/15E/A), and one from Endamohoni (T-023/08MW) were selected for this study in controlled condition by priming in four salt treatment levels (5 dS/m, 7 dS/m, 9 dS/m and 15 dS/m). Distilled water (0 dS/m) was used as control. Fifty (50) surface sterilized seeds per Petri dish were sown for the four salt treatments and the control tests. Randomized complete block design (RCBD) with four replications was used throughout the culture. Four replications of 50 seeds of P. sativum var. abyssinicum for each accession were grown on Whatman No. 2 filter papers (Fig. 1) with 50 ml of respective treatment solutions. The salt solution watering was twice per day in the early morning and late evening. The growth media were put in a sealed polythene bags to prevent evaporation. Seeds were allowed to germinate and grow at (24±1) °C for 14 days. Root growth features, shoot growth traits were examined and measured. The mean germination time was calculated for the rate of germination with the following formula (Kandil et al., 2012):

$$MGT = \frac{\sum dn}{\sum n}$$

Where, n is the number of seeds which were germinated at day 'd', and d is the number of days counted from the beginning of sowing. Germination index (GI) was calculated as the product of number of days after sowing (Li, 2008), and number of germinated seeds divided to the total number of sown seeds for the correlation analysis.

$$GI = \frac{\sum diNi}{s}$$

Where, 'di' is number of days after sowing of seeds, 'Ni' is number of germinated seeds, 'S' is total number of sown seeds. Root length, shoot length, and seedling fresh weights were measured on the 14<sup>th</sup> day. Fifteen grams of seeds from each accession of *P. sativum* var. *abyssinicum* were placed in Petri dishes containing distilled water to determine water uptake of seeds necessary for germination. The water uptake was measured as fresh weight percentage increase in seed weight.



**Fig. 1:** Fifty seeds of *P. sativum* var. *abyssinicum* for each accession plated in growth chambers (Petri dishes).

**Table 1.** Description by sources of regions, districts and altitudial variation of the 30 Dekoko accessions studied and the six better performers selected for salt resistances.

Code	and name of Accessions	Description of sources area						
No Name of Accession		Name of region	Name of district	Agro-ecology	Altitude (m.a.s.l)			
1	T-025/15E/A	Tigray	Emba-Alaje	highland	2116			
2	TA-026/15Sr	Amhara	Sirinka	Midland	1868			
3	T-001/08OF	Tigray	Ofla	highland	2457			
4	T-023/08MW	Tigray	Endamohoni	highland	2100			
5	T-002/04OF	Tigray	Ofla	highland	2457			
6	T-003/04OF	Tigray	Ofla	highland	2457			
7	TK-004/08AL	Tigray	Alamat	lowland	1178-3148			
8	TK-005/08AL	Tigray	Alamata	lowland	1178-3148			
9	TK-006/08AL	Tigray	Alamat	lowland	1178-3148			
10	TK-008/08AL	Tigray	Alamat	lowland	1178-3148			
11	T-022/08E/A	T igray	Emba-Alaje	highland	2116			
12	T-024/08E/A	Tigray	Emba-Alaje	highland	2116			
13	T-021/08H/W	Tigray	Hintalo-Wajerat	midland	1400-3050			
14	T-007/08KO	Amhara	Kobo	lowland	1100-3000			
15	T-009/08KO	Amhara	Kobo	lowland	1100-3000			
16	T-010/08KO	Amhara	Kobo	lowland	1100-3000			
17	T-017/08KO	Amhara	Kobo	lowland	1100-3000			
18	T-018/08KO	Amhara	Kobo	lowland	1100-3000			
19	T-019/08KO	Amhara	Kobo	lowland	1100-3000			
20	T-020/08KO	Amhara	Kobo	lowland	1100-3000			
21	T-012/08G/L	Amhara	Guba-lafto	highland	2061			
22	TA-013/08Sr	Amhara	Sirinka	midland	1868			
23	TA-014/08Sr	Amhara	Sirinka	midland	1868			
23	TA-015/08	Amhara	Sirinka	midland	1868			
25	T-011/08Ha	Amhara	Habru	lowland	700-1900			
26	T-016/08hA	Amhara	Habru	lowland	700-1900			
27	T-027/15Sr	Amhara	Sirinka	midland	1868			
28	T-028/15MW	Tigray	Endamehoni/mychew	highland	2100			
29	T-029/15MW	Tigray	Endamehoni/mychew	highland	2100			
30	T-030/15E/A	Tigray	Emba- Alaje	highland	2116			

**Note:** Accessions written in bold (1-6) were target accessions for the resistance/adaptation study.

#### **Results and discussion**

The results in Table 2 show that there is statistically significant difference on the seedling growth traits among the six *P. sativum* var. *abyssinicum* accessions. This indicates the existences of considerable variability among the accessions for most of the growth parameters after salt treatments. This implies that, the accessions even may belong to the same variety (species) but their cultivation in different areas force them to have different adaptation mechanisms for abiotic stress like in this case salinity. This lead to different cellular adaptive responses such as accumulation of compatible solutes as the salt priming of seeds prior to sowing enhances a process used

naturally by plants to minimize the movement of Na<sup>+</sup> to the shoot because of the complexity of interactions between stress factors and various molecular, biochemical and physiological phenomena affecting plant growth and development among the accessions (Hamdia and Shaddad, 2010; Tsegay and Gebreslassie, 2014). T-025/15E/A, TA-026/Sr, T-023/08MW, T-001/08OF perform better in descending order for root growth features with root height reduction percentage of negative value at the medium salt concentrations (Table 2) indicating increases in root length from the untreated seeds for the increases of Na<sup>+</sup> content of the root systems the need to accumulate inorganic and organic solutes for resisting osmotic stress induced by various salt stresses (Wang et al., 2015).

Table 2. The effect of NaCl treatment on Pisum sativum var. abyssinicum growth parameters (root length, root fresh and dry weights) under salinity stress.

Treatments	Accessions water uptake (%)					Accessions germination (%)							
(NaCl)	T- 001/08OF	T- 002/08OF	T- 003/08OF	TA- 026/15Sr	T- 025/15E/A	T- 023/08MW	T- 001/08OF	T-002/08OF	T-003/08OF	TA-026/15Sr	T-025/15E/A	T-023/08MW	
0dS/m	55.43 <sup>a</sup>	56.24 <sup>a</sup>	54.79 <sup>c</sup>	60.9°	63.89 <sup>c</sup>	59.16 <sup>ab</sup>	82.04 <sup>a</sup>	79.54 <sup>b</sup>	67.08 <sup>a</sup>	83.86 <sup>a</sup>	90.00 <sup>a</sup>	78.32 <sup>a</sup>	
5dS/m	$70.98^{a}$	69.45 <sup>a</sup>	$71.00^{b}$	$78.90^{b}$	80.01 <sup>b</sup>	84.23 <sup>ab</sup>	57.68 <sup>b</sup>	48.54 <sup>c</sup>	37.87 <sup>b</sup>	81.22 <sup>a</sup>	88.78 <sup>a</sup>	81.44 <sup>a</sup>	
7dS/m	78.67 <sup>a</sup>	$75.06^{a}$	73.46 <sup>a</sup>	$82.20^{b}$	86.96 <sup>a</sup>	75.05 <sup>ab</sup>	34.55 <sup>c</sup>	18.67 <sup>d</sup>	28.32°	$65.00^{b}$	76.02 <sup>b</sup>	61.61 <sup>b</sup>	
9dS/m	82.30 <sup>a</sup>	83.01 <sup>a</sup>	$84.70^{a}$	89.22 <sup>a</sup>	90.43 <sup>a</sup>	85.67 <sup>ab</sup>	17.95 <sup>d</sup>	01.65 <sup>e</sup>	25.22 <sup>d</sup>	55.08 <sup>b</sup>	65.54 <sup>b</sup>	49.00°	
TD 4	Accessions germination rate							Accessions mean germination time (days)					
Treatments (NaCl)	T- 001/08OF	T- 002/08OF	T- 003/08OF	TA- 026/15Sr	T- 025/15E/A	T- 023/08MW	T- 001/08OF	T-002/08OF	T-003/08OF	TA-026/15Sr	T-025/15E/A	T-023/08MW	
0dS/m	13.28 <sup>a</sup>	13.85 <sup>a</sup>	11.68 <sup>a</sup>	14.60 <sup>a</sup>	15.67 <sup>a</sup>	13.16 <sup>a</sup>	3.1°	3.21 <sup>c</sup>	3.41°	4.63 <sup>a</sup>	4.08 <sup>a</sup>	3.61 <sup>b</sup>	
5dS/m	14.04 <sup>a</sup>	8.45 <sup>b</sup>	6.59 <sup>c</sup>	14.74 <sup>a</sup>	15.86 <sup>a</sup>	14.66 <sup>a</sup>	$3.70^{b}$	3.64 <sup>b</sup>	2.92 <sup>c</sup>	$4.00^{a}$	4.22 <sup>a</sup>	3.72 <sup>b</sup>	
7dS/m	6.01 <sup>c</sup>	3.25 <sup>e</sup>	4.93 <sup>c</sup>	11.32 <sup>b</sup>	13.24 <sup>b</sup>	10.73 <sup>b</sup>	4.01 <sup>a</sup>	$3.89^{b}$	2.43 <sup>d</sup>	4.55 <sup>a</sup>	4.86 <sup>a</sup>	$4.02^{a}$	
9dS/m	$3.12^d$	$0.29^{d}$	4.39 <sup>d</sup>	9.59 <sup>b</sup>	11.42 <sup>b</sup>	8.53 <sup>b</sup>	$0.89^{d}$	$0.65^{d}$	$0.00^{e}$	1.57 <sup>d</sup>	$3.00^{b}$	$2.50^{\rm c}$	
T44	Accessions root length (cm)						Accessions root height reduction (%)						
Treatments (NaCl)	T- 001/08OF	T- 002/08OF	T- 003/08OF	TA- 026/15Sr	T- 025/15E/A	T- 023/08MW	T- 001/08OF	T-002/08OF	T-003/08OF	TA-026/15Sr	T-025/15E/A	T-023/08MW	
0dS/m	9.31 <sup>a</sup>	9.46 <sup>a</sup>	11.03 <sup>a</sup>	12.11 <sup>a</sup>	12.59 <sup>b</sup>	9.87 <sup>a</sup>	$0.00^{e}$	$0.00^{c}$	$0.00^{d}$	$0.00^{b}$	$0.00^{a}$	$0.00^{c}$	
5dS/m	10.04 <sup>a</sup>	$9.73^{a}$	7.93 <sup>b</sup>	13.23 <sup>a</sup>	14.22 <sup>a</sup>	10.32 <sup>a</sup>	-7.84 <sup>d</sup>	-2.85 <sup>d</sup>	28.11 <sup>c</sup>	-9.25 <sup>d</sup>	-12.95 <sup>d</sup>	-4.56 <sup>d</sup>	
7dS/m	8.13 <sup>b</sup>	$7.00^{b}$	6.81 <sup>b</sup>	12.42 <sup>a</sup>	13.66 <sup>a</sup>	7.51 <sup>b</sup>	12.68 <sup>b</sup>	$26.00^{b}$	38.26 <sup>b</sup>	-2.26 <sup>c</sup>	-8.50°	23.91 <sup>b</sup>	
9dS/m	1.46 <sup>c</sup>	1.50°1.01°		5.20 <sup>b</sup>	5.50 <sup>b</sup>	$0.99^{c}$	84.34 <sup>a</sup> 84.14	1 <sup>a</sup>	90.84 <sup>a</sup>	57.06 <sup>a</sup>	56.31 <sup>b</sup>	89.97 <sup>a</sup>	
T4	Accessions root fresh weight (g)  Accessions root dry weight(g)												
Treatments (NaCl)	T- 001/08OF	T- 002/08OF	T- 003/08OF	TA- 026/15Sr	T- 025/15E/A	T- 023/08MW	T- 001/08OF	T-002/08OF	T-003/08OF	TA-026/15Sr	T-025/15E/A	T-023/08MW	
0dS/m	6.40 <sup>b</sup>	5.62 <sup>b</sup>	5.43 <sup>b</sup>	6.67 <sup>b</sup>	6.58 <sup>b</sup>	6.51 <sup>b</sup>	3.04 <sup>b</sup>	2.34 <sup>b</sup>	2.28 <sup>a</sup>	3.50 <sup>a</sup>	3.52 <sup>b</sup>	2.74 <sup>a</sup>	
5dS/m	$7.30^{a}$	$7.40^{a}$	6.82 <sup>a</sup>	7.51 <sup>a</sup>	$7.49^{a}$	7.54 <sup>a</sup>	4.71 <sup>a</sup>	3.51 <sup>a</sup>	2.81 <sup>a</sup>	$3.42^{b}$	4.03 <sup>a</sup>	$2.60^{a}$	
7dS/m	5.92°	4.89 <sup>c</sup>	$5.40^{\rm b}$	7.88 <sup>a</sup>	$8.00^{a}$	7.22 <sup>a</sup>	$3.90^{a}$	2.01 <sup>c</sup>	1.87 <sup>b</sup>	$3.94^{a}$	4.21 <sup>a</sup>	1.87 <sup>b</sup>	
		$3.62^{d}$	3.01°	$6.20^{b}$	7.72 <sup>a</sup>	4.75°	$2.20^{\rm c}$	1.93 <sup>c</sup>	1.43°	2.73 <sup>c</sup>	3.52 <sup>b</sup>	1.06°	

Subscriptions (a-e) with different letters indicate significant difference among means within the columns (p<0.05, using LSD test).

The increase in water uptake percentage of the Pisum sativum var. abyssinicum accessions (Table 2) treated with salt (5 dS/m, 7 dS/m and 9 dS/m) compared with the control (0d S/m) indicated that physiological activities are enhanced for salt stress resistances induced by various salt stresses. These processes ensure that the water balance that can provide a relative normal physiological environment for the study accessions (Wang et al., 2015). Even among the six accessions there experienced a difference in water uptake percentage where by the better performing in germination percentage, germination rate, and root length required more that than the less performers; taking T-025/15E/A, TA-026/Sr, T-023/08MW, T-001/08OF the four medium salt treatment resistant accessions in descending order (Table 2). This means there is a considerable difference in percentage of seedling survivorship among the accessions (Wu and Lin, 2008).

The increases in mean germination time of the salt stress resistant promising accessions (T-025/15E/A, TA-026/Sr, T-023/08MW, T-001/08OF) is in relation to their germination stage tolerances to salt stress (Table 2). A negative relationship between germination percentage, and water uptake percentage and mean germination time was observed in the promising accessions of the control and different salt treatments. Seeds did not emerge when the salinity exceeds 9 dS/m where no seeds were germinated at the 15 dS/m treatment. The increases in mean germination time of the resistant/tolerant accession are because of the adaptive features of root elongation for water uptake search and survivorship mechanisms of the germinated seeds after stress (Ramoliya and Pandey, 2003). Similarly findings from Janagard et al. (2008) stated attributes associated to root and shoot to be the two most important features determining adaptation of plants to different salinity conditions. It is known that roots are the first organs that face the corrosion due to salinity affecting the whole growth features of the crop. Toxic effect of sodium chloride causes reduction in shoot growth through unbalanced nutrient uptake under the NaCl stress.

With respect to root fresh weight and dry weight at germination the resistant accessions of *Pisum sativum* var. *abyssinicum* have better value at the medium salinity stresses; 5 dS/m and 7 dS/m although salt stress is negatively affected growth and development of these accessions as it increases above 7 dS/m and even at the

medium salt treatments for the other accessions (Table 2). This is related to the root biomass relationship of the crop and the salinity tolerance of accessions increased because of the root Na<sup>+</sup> increments (Akinci et al., 2010).

Shoot growth parameters such as shoot length, shoot height reduction, shoot fresh weight and shoot dry weight were assayed (Fig. 2). T-025/15E/A followed by TA-026/15Sr and T-003/08OF with similar responses showed better seedling growth at 5 dS/m and the shortest shoot length at this salt treatment was observed in T-002/08OF. The highest shoot height reduction was observed from T-003/08OF accession at all the salt stress levels. This may be because of the accession is hampered by hyperhydricity a phenomenon of deformity in which the shoots become translucent during weight losing their original form and physiology within a short period of time (Wu et al., 2011).T-025/15E/A and TA-026/15Sr respectively are salt resistant accession in all the salinity treatments with respect to shoot length of the seedlings (Fig. 2). The reason is that, these accessions have lower concentration of Na<sup>+</sup> and Cl<sup>-</sup>concentrations in their shoots contrasting to the sensitive accessions (Lahir et al., 2009).

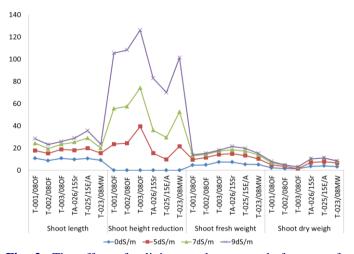


Fig. 2: The effect of salinity on shoot growth features of different Dekoko accessions.

Collected data was also subjected to total correlation analysis based on all the relevant variables. Total correlation analysis for salt stress adaptation showed no significant linear association among accessions, interaction between accession and salt concentration, and interaction between accession and growth features (Table 3). From the current study, salt concentration is negatively correlated with seed germination, shoot length, shoot height reduction, shoot fresh weight and

shoot dry weight i.e., as the salt concentration increased from 5 dS/m to 15 dS/m, the aforementioned variables decreased linearly. The longest shoot length was obtained at all treatments from T-025/15E/A followed by the T-001/08OF and TA-026/15Sraccessions (Fig. 2). Shoot length was severely influenced by salt stress with complete inhibition and no shoots grown at 15 dS/m NaCl stress in most of the accession but of with dwarf shoot from T-025/15E/A and TA-026/15Sr. This is because; increasing in osmotic potential (more negative) decreases the movement of nutrients and sap up and down the stem of the crop causes major reductions in crop productivity and quality. Salinity effects are the

results of complex interactions among morphological, physiological, and biochemical processes including seed germination, plant growth, and water and nutrient uptake (Shrivastava and Kumar, 2015).

Compared to control, each increase in NaCl concentration resulted in remarkable decrease in shoot fresh weight for all accessions (Fig. 2). The highest shoot fresh weight (100 g/plant) was observed from T-025/15E/A followed by accession TA-026/15Srand T-003/08OF at the control and 5 dS/m the lowest salt treatment (Fig. 2). However the distributions demonstrated a very different picture among the six accessions.

**Table 3.** Pearson correlation coefficient between accession and, salt concentration for *P. sativum* var. *abyssinicum* shoots growth parameters.

	2	3	4	5	6	7	8	9
1	0	0.217	-0.049	-0.049	-0.047	-0.249	0.008	-0.242
2		0.109	-0.926**	-0.962**	-0.952**	-0.818**	-0.901**	-0.807**
3			-0.173	-0.173	-0.205	-0.105	-0.212	-0.219
4				1.00**	$0.973^{**}$	$0.880^{**}$	-0.790**	0.818**
5					$0.973^{**}$	$0.880^{**}$	-0.790**	0.818**
6						$0.865^{**}$	-0.814**	$0.811^{**}$
7							-0.776**	$0.869^{**}$
8								-0.734**

(1) Accession; (2) Salt concentration (dS/m); (3) Water uptake percentage; (4) Seed germination; (5) Mean Germination time (days); (6) Shoot length (cm); (7) Shoot height reduction percentage; (8) Shoot fresh weight (gm); (9) Shoot dry weight (gm) after oven dry. \*\*. Correlation is significant at the 0.01 level (2-tailed).

**Table 4.** Pearson correlation coefficient of salt concentration, shoot growth with other *P. sativum* var. *abyssinicum* seedling growth traits.

	2	3	4	5	6	7	8	9
1	-0.952**	-0.818**	-0.901**	-0.807**	-0.888**	-0.880**	-0.902**	-0.759**
2		$0.865^{**}$	-0.814**	$0.811^{**}$	$0.856^{**}$	$0.874^{**}$	$0.845^{**}$	0.765**
3			-0.776**	$0.869^{**}$	$0.856^{**}$	$0.858^{**}$	$0.757^{**}$	0.635**
4				-0.734**	-0.784**	-0.789**	-0. 947 <sup>**</sup>	-0.801**
5					$0.897^{**}$	0.683**	0. 693**	$0.645^{**}$
6						$0.754^{**}$	$0.715^{**}$	$0.716^{**}$
7							$0.827^{**}$	$0.553^{*}$
8								$0.781^{**}$

Salt concentration (dS/m); (2) Shoot length (cm); (3) Shoot height reduction (%); (4) Shoot fresh weight; (5) Shoot dry weight after over dry; (6) Vigor index; (7) Leaf branch per leaf; (8) Germination index; (9) Relative NaCl injury rate. \*\*. Correlation is significant at the 0.01 level (2-tailed); \*. Correlation is significant at 0.05 levels (2-tailed).

As shown in Table 4, the effect of salt treatment is negatively correlated with vigor index, leaf branch, germination index and relative NaCl injury rate. The relative measure of standardized variables of *P. sativum* var. *abyssinicum* seedling growth traits showed a decreased trend with increasing NaCl salt concentration. The highest coefficient of correlation was observed from vigor index followed by leaf branches. The decrease in number of branches per plant of *P. sativum* var.

abyssinicum may be to decrease the leaf injury by adjusting high leaf k<sup>+</sup>/Na<sup>+</sup> ratio and having fewer branches instead of branches that aggravate leaf injury due to its lose husk. This is in line with the finding of Shahid et al. (2013) where number of branches decreased with salt treatment in pea cultivars.

From overall correlation analysis (data not shown), the correlation was significant (p<0.01 and p<0.05) between

NaCl salt treatments and *P. sativum* var. *abyssinicum* seedling growth traits. NaCl stress has negatively affected shoot length, shoot height reduction, shoot fresh weight, and shoot dry weight after oven dry as a result of other seedling growth traits such as vigor index, leaf branch per plant, germination index and relative NaCl injury rate.

#### **Conclusion**

It can be concluded that since accessions of Pisum sativum var. abyssinicum are collected from different attitudinally variable agro-ecologies, they have different adaptive responses to salt stress indicating the same subspecies growing in different areas vary to abiotic stress responses such as salinity. Moreover, Pisum sativum var. abyssinicum is salt tolerant more at germination stage than seedling stage. On the basis of the results of this work also, it is clear that seed germination, root length, shoot length have significant association with salt stress; the more the salinity, the more reduction in these growth performances. But, T-023/08MW, T-001/08OF, TA-026/15Sr followed by T-025/15E/A from lower to the higher resistances respectively could withstand lower to medium concentrations of salinity as compared to the other accessions similar to what has been found from the field and wire-house trial of these accessions.

#### **Conflict of interest statement**

Authors declare that they have no conflict of interest.

### Acknowledgement

The author is grateful to Dr. Berhanu Abraha for helping in the seed samples collection and the paper evaluation before submission, Senait Sisay for helping checkup of the coherence and consistency of the references for organized collection of the whole references. Research funding from the Ethiopian Minister of Education, Bahir Dar University and Woldia University are gratefully acknowledged. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

#### References

Akinci, I.E., Akinci, S., Yilmaz, K., Dilici, H., 2010. Response of eggplant varieties (*Solanum melongena*) to salinity in germination and seedling stages. New Zeal. J.

- Crop Hort. Sci. 32, 193-210.
- Ashraf, M., Foolad, M.R., 2007. Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ. Exp. Bot. 59, 206-216.
- Barpete, S., Aasim, M., Khawar, M. K., Özcan, S.F., Özcan, S., 2014. Preconditioning effect of cytokinins on *in vitro* multiplication of embryonic node of grass pea (*Lathyrus sativus* L.) cultivar Gürbüz. Turk. J. Biol. 38, 485-492.
- Berhane, G., Berhanu, A., 2016. Review: Distribution and productivity of Dekoko (*Pisum sativum* var. *abyssinicum* A. Braun) in Ethiopia. Global J. Sci. Front. Res. 16, 45-57
- CSA, 2011. Agricultural Sample Survey: Report on Area and Production for Major Crops (Private Peasant Holdings, Meher Season). Central Statistical Authority, Addis Ababa, Ethiopia.
- Esquinas-Alacazar, J., 2005. Protecting crop genetic diversity for food security: political, ethical and technical challenges. Nature Rev. Genet. 6, 946-953.
- FAO, 2012. FAOSTAT. Food and Agriculture Organization of the United Nations, Rome, Italy.
- Girmay, T., Girmay, G., Alem, G., Abrhaley, G., Yemane, G., Tesfay, H., 2014. Participatory Rural Appraisal Report: Raya-AzeboWoreda, Tigray Region.
- Haddis, Y., Dargie, T., 2013. Characterization of Dekoko (*Pisum sativum* var. *abyssinicum* A.Bruan) accessions by qualitative traits in the highlands of Southern Tigray, Ethiopia. Afr. J. Plant Sci. 7, 482-487.
- Hamdia, M. A., Shaddad, M. A. K., 2010. Salt tolerance of crop plants. J. Stress Physiol. Biochem. 6(3), 64-90.
- Hu, Y., Schmidhalter, U., 2002. Limitation of salt stress to plant growth. In: Plant Toxicology (Eds.: Hock, B., Elstner, C.F.). Marcel Dekker Inc., New York. pp.91-224.
- Janagard, MS., Tobeh, A., Esmailpour, B., 2008. Evaluation of salinity tolerance of three canola cultivars at germination and early seedling growth stage. J. Food Agric. Environ. 6, 272-275.
- Kandil, A.A., Sharief, A.E., Ahmed, S.R.H., 2012. Germination and seedling growth of some chickpea cultivars (*Cicer arietinum* L.) under salinity stress. J. Basic Appl. Sci. 8, 561-571.
- Keneni, G., Assefa, F., Imtiaz, M., Bekele, E., 2013. Genetic diversity for attributes of biological nitrogen fixation in Abyssinian field pea (*Pisum sativum* var. *abyssinicum*) germplasm accessions. Ethiop. J. Appl. Sci. Technol. 4, 1-20.
- Lahir, A. N., Garg, B.K., Vyas, S.P., Kathju, S., Mali, P.C., 2009. Genotypic differences to soil salinity in cluster bean. Arid Soil Res. Rehabilit. 10, 333-345.
- Li, Y., 2008. Effect of salt stress on seed germination and seedling growth of three salinity plants. Pak. J. Biol. Sci. 11, 1268-1272.
- Munns, R., Tester, M., 2008. Mechanisms of salinity tolerance. Annu. Rev. Plant Biol. 59, 651-681.
- Ramoliya, P. J., Pandey, A. N., 2003. Soil salinity and water status affect growth of *Phoenix*

- dactylifera seedlings. New Zeal. J. Crop Hort. Sci. 31, 345-353.
- Shahid, M. A., Ashraf, M.Y., Aslampervez, M., Ahmad, R., Mukhtarbalal, R., Garcia-sanchez, F., 2013. Impact of salt stress on concentrations of Na<sup>+</sup>, Cl<sup>-</sup> and organic solutes concentration in pea cultivars. Pak. J. Bot. 45, 755-761.
- Shrivastava, P., Kumar, R., 2015. Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. Saudi J. Biol. Sci. 22, 123-131.
- Smýkal, P., Coyne, C.J., Ambrose, M.J., Maxted, N., Schaefer, H., Blair, M.W., Varshney, R.,K., 2015. Legume crops phylogeny and genetic diversity for science and breeding. Crit. Rev. Plant Sci. 34, 43-104.
- Tsegay, B.A., Gebreslassie, B., 2014. The effect of salinity (NaCl) on germination and early seedling growth of *Lathyrus sativus* and *Pisum sativum* var. *abyssinicum*.

- Afr. J. Plant Sci. 8, 225-231.
- Wang, L., Fang, C., Wang, K., 2015. Physiological responses of *Leymus chinensis* to long-term salt, alkali and mixed salt-alkali stresses. J. Plant Nutr. 38, 526-540.
- Wild, A., 2003. Soils, Land and Food: Managing the Land during the Twenty-first Century. Cambridge University Press, Cambridge, UK.
- Wu, H.J., Yu, X., Teixeira da Silva, J. A., Lu, G.P., 2011. Direct shoot induction of *Paeonia lactiflora* 'Zhong Sheng Fen' and rejuvenation of hyperhydric shoots. New Zeal. J. Crop Hort. Sci. 39, 271-278.
- Wu, L., Lin, H., 2008. Salt tolerance and salt uptake in diploid and polyploidy buffalo grasses (*Buchloe dactyloides*). J. Plant Nutr. 17, 1905-1928.
- YemaneA., Skjelvåg, A.O., 2002. The physico-chemical features of Dekoko (*Pisum sativum* var. *abyssinicum*) seeds. J. Agron. Crop Sci. 189, 14-22.

#### How to cite this article:

Berhane, G. G., Berhanu, A. T., 2017. Adaptability of Dekoko (*Pisum sativum* var. *abyssinicum*) seedlings to salinity stress in *in vitro* culture conditions. Int. J. Curr. Trend. Pharmacobiol. Med. Sci. 2(1), 16-24.