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EFFECTS OF SALINITY STRESS ON SEEDLING GROWTH OF LOCAL VARIETIES OF FABA BEAN (*VICIA FABA* L.) FROM INDIA

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ABSTRACT

Two local faba bean varieties (Va and Vb) were evaluated for salt tolerance under different salinity treatments i.e. EC_3, EC_6, EC_9, EC_{12}, EC_{15}, EC_{18} and EC_{25} (dS/m) along with control (EC_0) in present study. Seed germination and early seedling growth & biomass characteristics (plumule, radical and total seedling length, fresh and dry weight of seedlings), seedling vigor (SV) and salinity stress index (Tsegay and Gebreslassie) were measured. The result showed that all the traits decreased gradually with increase in salinity in both varieties with significant variations but this decrease was less in variety V(a) than variety V(b). Seed germination was less affected than the seedling growth characteristics. Less reduction in radical length than the plumule length showed greater adaptations of the plants under saline environment. Performance of V(a) was found to be more significant in comparison to V(b) even at high salinity. Therefore, present study suggests that V(a) is more resistant towards salinity. This can be used to study salinity tolerance mechanisms and pathways in more detail in legumes. Screening of large number of crop genotypes salt tolerance may also provide promising source for breeding program. Selected salt tolerant varieties can also be recommended for salinity prone areas as management strategy.

Keywords: Faba Bean, Salinity stress, Seed germination, Seedling growth, Seedling vigor, Salinity stress index.

Introduction

Faba bean (*Vicia faba* L.), commonly known as field bean, is an important cool season annual legume with high nutritive value (Karkanis *et al.*, 2018). It is the third important leguminous plant after soyabean and peas (Turco *et al.*, 2016). Faba bean seeds are rich source of proteins, carbohydrates, mineral nutrients (K, Ca, Mg, Fe, and Zn), vitamins, and fiber content (Neme *et al.*, 2015). Therefore, the fresh and dry seeds of faba bean are used for human consumption. This crop has also pharmacological importance due to presence of considerable amount of different bioactive compounds (Landry *et al.*, 2016; Turco *et al.*, 2016). It improves soil properties and fertility due to highly efficient symbiotic association of its roots with soil *Rhizobium* responsible for atmospheric nitrogen fixation (Adak and Kibritci, 2016; Argaw and Mnalku, 2017). Faba bean is also an important legume in various cropping systems as rotation or break crop. Moreover, incorporation of legumes into the soil as green manure increases the growth and yield of several crops (Zou *et al.*, 2015). The global production of faba bean grain was 4.1 million tons in 2014 (FAO, 2017).

Production of legumes including faba bean is severally affected due to their susceptibility towards existing environmental factors i.e. abiotic and biotic stresses (Singh *et al.*, 2012). Among abiotic stress factors, salinity stress is a major threat for plant production, in arid and semi-arid regions, particularly in irrigated agriculture (Fuskhah *et al.*, 2019; Sehrawat *et al.*, 2019; Sehrawat *et al.*, 2020a). Continuous increase in salinity (1-3% every year) raises serious concerns for nutritious plant production due to conversion of a large proportion of arable land into saline or non-fertile land (Qadir *et al.*, 2014; Sehrawat and Yadav, 2020; Sehrawat *et al.*, 2020b). Salinity affects the plant growth and development throughout their life time including seed germination, seedling growth, vegetative growth, reproductive phase, maturity and grain yield i.e. quality and quantity (Sehrawat *et al.*, 2014c; Sehrawat *et al.*, 2014b; Sehrawat *et al.*, 2014a; Sehrawat & Yadav, 2014d, 2020; Sehrawat *et al.*, 2015; Sehrawat *et al.*, 2019; Sehrawat *et al.*, 2020b). Excess amount of salt in soil suppress the growth, nitrogen fixation ability, nodulation and yield in faba bean plants (Abd-Alla *et al.*, 2001; Bulut *et al.*, 2011; Katerji *et al.*, 2011). During initial stage, salinity stress either inhibits or delay seed germination because it affect the activity of the enzymes involved in digestion of complex stored food material in seed (Ma *et al.*, 2017; Panda, 2001; Sehrawat *et al.*, 2014a). After seed germination, salinity stress arrest proper growth and survival of seedlings. But the plants exhibited less adverse effect on germination and seedling growth under salinity show salt tolerance (Sehrawat *et al.*, 2016).

Salinity is a continuous increasing problem for agriculture plant production in current scenario due to global climate change and abnormal irrigation practices (Fuskhah *et* *al*., 2019; Nadeem *et al*., 2019; Sehrawat and Yadav, 2020; Sehrawat *et al*., 2020b). Salinity adversely affects the plant growth, physiology and metabolism but different crop species respond variably towards salinity during each developmental stage throughout their life cycle (Sehrawat *et al*., 2013a; Sehrawat *et al*., 2014a; Sehrawat *et al*., 2015). Crop based management of salinity is more efficient and economic approach to alleviate salinity as compared to the soil and irrigation management strategies which are highly expensive (Sehrawat *et al*., 2020b). Development of genetically improved variety/lines needs presence of significant variations in crop plants for salt tolerance at genetic level (Sehrawat *et al*., 2014b; Sehrawat *et al*., 2016). This requires screening of crop varieties or available germplasm for salt tolerance during different growth stages (Sehrawat *et al*., 2014c; Sehrawat *et al*., 2013b; Sehrawat *et al*., 2015). However, very little efforts have been made towards screening of faba bean genotypes for salt tolerance (Karaköy *et al*., 2014). Local landraces or wild relatives constitute co-adapted genes for desired traits that may prove valuable in future cultivation practices to enhance grain yield and quality (Sehrawat *et al*., 2013c; Sehrawat and Yadav, 2014d; Sehrawat *et al*., 2019; Shanko *et al*., 2017). Therefore, the local crop varieties represent an important source for breeding programs (Harlan, 1975). Lack of suitable technique and reliable parameters for salt tolerance further restricts suitable efforts made in this area (Sehrawat *et al*., 2019).

Seed germination is the first stage encounters salinity stress. Saline environment may create an osmotic potential external to the seed preventing sufficient water uptake during imbibition for seed germination. Toxic effects of sodium and chloride ions may also inhibit germination of seeds (Hosseini *et al*., 2003; Kumar, 2017). Hence, salinity induced osmotic stress either inhibit or delay seed germination, emergence of seedling and seedling establishment (Bayuelo-Jiménez *et al*., 2002; Waheed *et al*., 2006). Rate of seed germination and emergence of seedling (radical and plumule) is an important determinant of successful crop establishment (Shu *et al*., 2017; Tsegay and Gebreslassie, 2014). Seedling vigor is an important criterion to determine salt tolerance in crop plants (Sehrawat *et al*., 2014c; Sehrawat *et al*., 2014b; Sehrawat *et al*., 2014a; Sehrawat and Yadav, 2014d; Sehrawat *et al*., 2014e, 2016). Moreover, maintenance of high salinity stress index during seedling growth stage results in greater adaptation and successful establishment of plants under salinity (Jamil *et al*., 2006; Sehrawat *et al*., 2016).

Keeping all these important facts in consideration, two local varieties of faba bean were investigated for salt tolerance during seed germination and seedling establishment under different level of salinity in present study. An effective level of salinity was also determined in this study that can be used to screen large number of genotypes for salt tolerance in rapid manner.

Material and Methods

Plant material and salinity stress levels

Seeds of two varieties of *Vicia faba* were used in this study (Figure 1). One variety (Va) was procured from main bazar, old subzi mandi, Delhi and second variety (Vb) from local market, Shahabad, Haryana. A total of seven salinity treatments i.e. EC_3, EC_6, EC_9, EC_{12}, EC_{15}, EC_{18} and EC_{25} (dS/m) along with control treatment (EC_0) were used. Different salinity levels were prepared by dissolving NaCl in water used for irrigation to impose salinity stress in faba bean plants under investigation during seed germination and early seedling growth. The control treatment was without salt (Sopha *et al*., 1991).

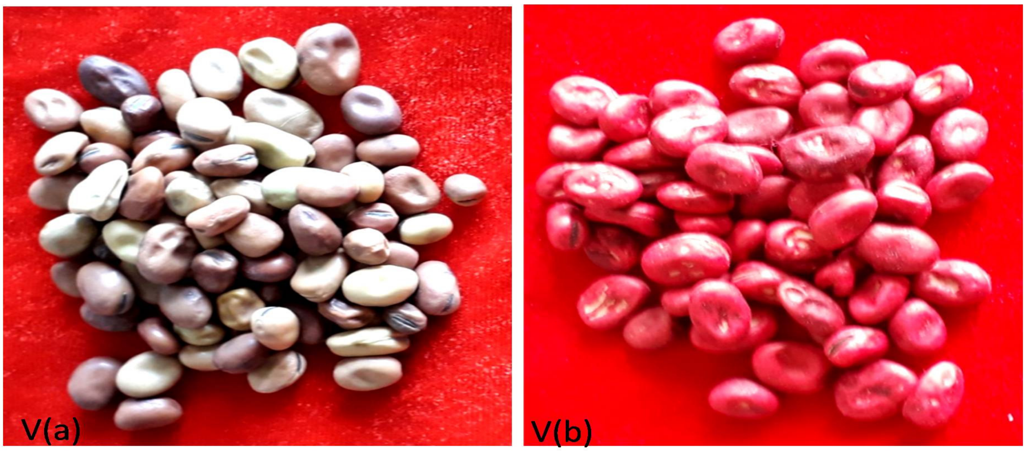

Fig. 1: Faba bean varieties; V(a) and V(b) used for salt tolerance study.

Method used

The seeds of both varieties of faba bean were allowed to germinate on filter paper soaked with 10 ml of saline solution of different concentrations i.e. EC_3, EC_6, EC_9, EC_{12}, EC_{15}, EC_{18} and EC_{25} (dS/m) along with their control treatment (EC_0). The experiment was carried out in the petridishes having five seeds in each for all treatments (control as well as salinity) with four replications, mean temperature of 25 ± 2°C and the relative humidity more than 60% under 16h photoperiod of cool fluorescent light. Seed germination percentage (SG %) and seedling growth & biomass characteristics (plumule, radical and total seedling length, fresh weight of seedlings) were measured on 11th day of the experiment. After taking fresh weight, the samples were dried in hot air oven at 60°C for 2 days. Then dry weight of the seedlings was recorded. The seedling vigor (SV) and salinity stress index (Tsegay & Gebreslassie, 2014) were calculated by using the formula i. e. SV = (Seed germination percentage x Total seedling length); SSI = [(Dry wt. of salt stressed seedling/dry wt. of control seedling) x 100]

(Sehrawat *et al.*, 2014e, 2016; Sopha *et al.*, 1991). Optimization of an effective salinity level was also done that can be used for further screening of more genotypes for salt tolerance during later growth stages. All the parameters were recorded with three replications per treatment and compared with their respective control. The recorded data was further subjected to statistical analysis of variance (ANNOVA) appropriate to the experimental design using OPSTAT program (HAU, Hisar, India) and the differences between the means were compared through least significant difference (LSD) test (P < 0.05).

Results

Present findings showed gradual reduction in seed germination and seedling growth characteristics with increase in salinity in comparison to the control in both varieties of faba bean. But at high salinity, either complete inhibition of seed germination or poor germination was recorded in this study. The investigated plants showed considerable variations for all the measured traits at different salinity levels as well as within themselves.

Table 1: Average value of all the observations recorded at different salinity levels in two local varieties of faba bean, *Vicia faba*.

Characters	Salinity Treatments (ds/m)								Critical Difference (CD) *		
	EC0	EC3	EC6	EC9	EC12	EC15	EC18	EC25	Treatment	Genotypes	Interaction (T x G)
Seed Germination (%)	100	100	100	100	100	100	80	60	1.44	2.15	5.09
Radical Length (cm)	5.20	4.58	4.12	3.00	2.33	1.63	1.22	0.32	0.071	0.119	0.228
Plumule Length (cm)	5.72	4.62	4.17	3.20	2.47	1.78	0.80	0.13	0.081	0.142	0.324
Total Seedling Length (cm)	10.92	9.20	8.28	6.20	4.80	3.42	2.02	0.45	0.121	0.194	0.411
Fresh wt. (mg) / seedling	2.03	1.88	1.67	1.67	1.53	1.44	1.18	1.13	0.016	0.027	0.061
Dry wt. (mg) / seedling	0.72	0.66	0.59	0.55	0.53	0.47	0.41	0.39	0.0019	0.004	0.010
Seedling Vigor Index	1091.33	920.00	828.33	620.00	480.00	305.73	161.33	31.00	12.06	18.42	36.14
Salt Stress Index	100	91.71	81.78	76.11	73.60	65.05	57.00	54.37	0.029	0.038	0.067

(*Significant value and p-value ≤ 0.01)

Seed germination percentage (SG %)

The result showed decrease and delay in seed germination with increase in salinity in both varieties (Figure 2; Table 1) but this reduction was less in variety (a) as compared to the variety (b). Variety (a) showed full germination up to salinity of EC15 but get reduced at high salinity (EC25) as compared to the variety (b) where seeds either fail to germinate or poorly germinated (Figure 2 and Figure 3). The results showed significant variations for seed germination among both varieties of faba bean and also at different salinity levels (Table 1). Seed germination affected the seedling growth in both varieties (Figure 4). The average percent reduction in seed germination due to salinity treatments was 8.57 % and 11.43% in V(a) and V(b) respectively as compared with their respective control. This shows greater tolerance of V(a) towards salinity stress and high susceptibility of V(b) in present study (Figure 5).

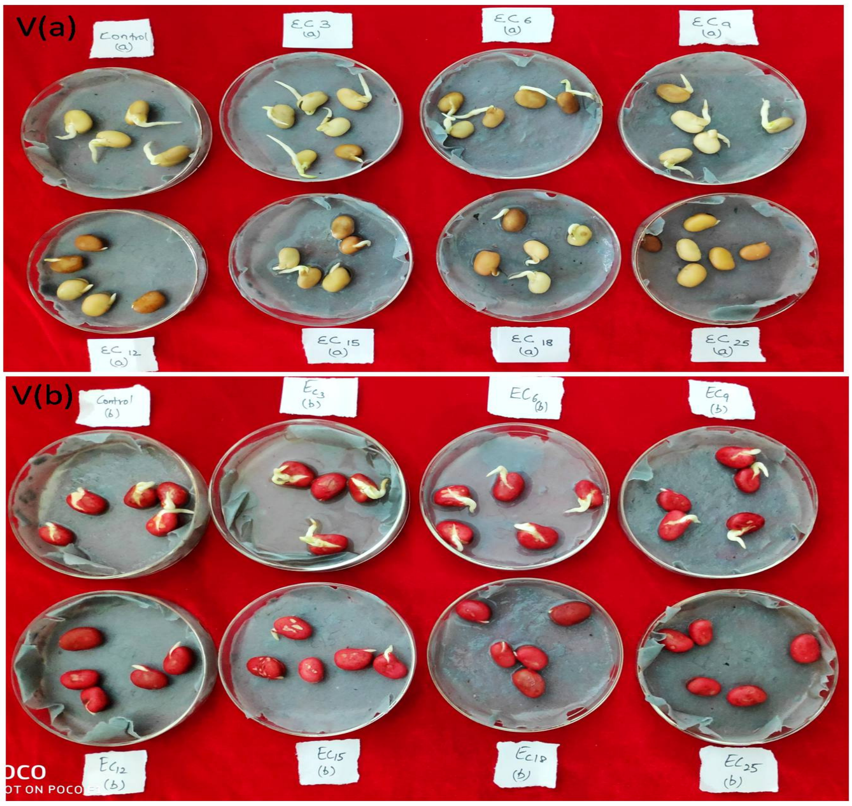

Fig. 2: Seed germination in faba bean varieties [V(a) and V(b)] under different salinity treatments [EC0; Control, EC3, EC6, EC9, EC12, EC15, EC18 and EC25 (dS/m)].

Seedling growth characteristics

The results showed that the seedling growth of faba bean was affected negatively due to salinity treatment. The total seedling length showed gradual reduction with increase in salinity over control in both faba bean varieties however, significant variations were observed in their response (Table 1; Figure 4). The observed reduction in seedling length was due to decrease in radical and plumule length with increasing salinity (Table 1). In present study, the percent reduction in radical length ranged from 7.10 % to 87.74 % in V(a) and from 16.56 % to 100 % in V(b) from low salinity (EC3) to high salinity (EC25) as compared with their respective control. Similarly, the plumule length get reduced from 20 % to 94 % in V(a) and from 22.83 % to 100 % in V(b) from low to high salinity. The percent reduction in total seedling length ranged from 14.12 % to 92.06 % and from 17.46 % to 100 % in V(a) and V(b) respectively at low and high salinity over control (Figure 5). But this decrease in seedling overall growth was recorded less for V(a) and greater for V(b). These findings demonstrated that V(a) is more resistant towards salinity than the V(b) which showed reverse response.

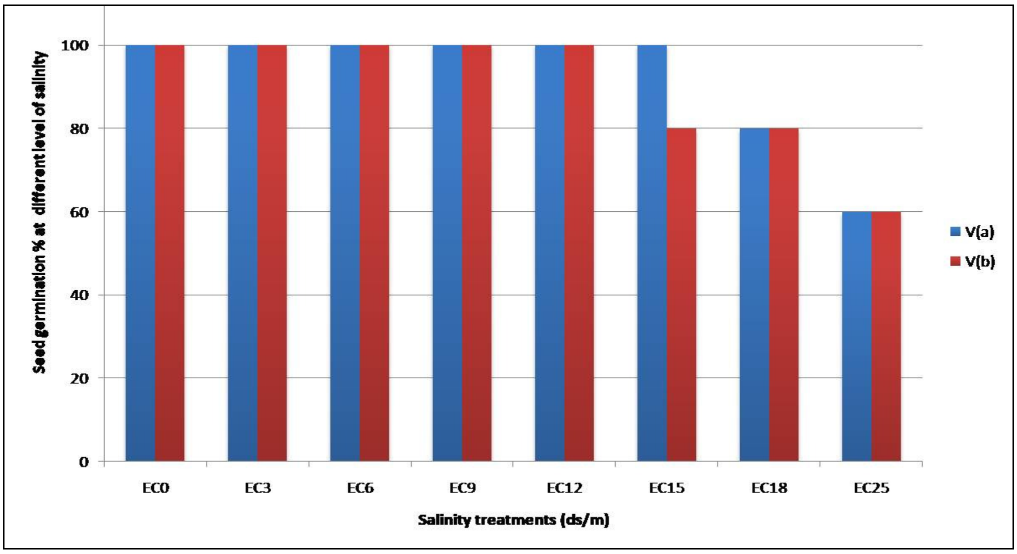

Fig. 3: Seed germination percentage of faba bean varieties [V(a) and V(b)] under different salinity treatments [EC0; Control, EC3, EC6, EC9, EC12, EC15, EC18 and EC25 (dS/m)].

Seedling biomass

The results showed that the biomass of seedlings in term of fresh and dry weight was reduced with increasing salinity in both varieties of faba bean however, this reduction was less in V(a) as compared to V(b) (Table 1; Figure 5). The percent reduction in fresh weight of the seedling over control ranged from 3.28 % to 24.71 % in V(a) and from 10.34 % to 58.90 % in V(b) from lower to higher salinity treatment (Figure 5). Whereas the reduction in dry weight of the seedling was from 7.92 % to 41.26 % in V(a) and from 8.66 % to 50 % in V(b) from lower to higher salinity over non-stressed seedlings. Maintenance of less reduction in seedling biomass by V(a) showed its greater resistance towards salt stress than V(b) which showed reverse response (Figure 5).

Fig. 4: Seedling growth of faba bean varieties [V(a) and V(b)] under different salinity treatments [EC0; Control, EC3, EC6, EC9, EC12, EC15, EC18 and EC25 (dS/m)].

Seedling vigor (SV)

The seedling vigor was also decreased with continuous increase in salinity in both faba bean varieties (Table 1). The reduction in seedling vigor ranged from 14.07 % to 94.53 % in V(a) and from 17.14 % to 100 % in V(b) from low (EC_3 ds/m) to high salinity (EC_{25} ds/m) as compared to the control having no salt. This reduction was due to decrease in seed germination and seedling growth under all salinity treatments in both varieties. However, the recorded response was also different at variety as well as salinity level (Figure 5). In this study, V(a) tried to maintain higher seedling vigor even at high salinity which showed its salt resistance behavior than V(b) which showed salt sensitive behavior (Figure 5).

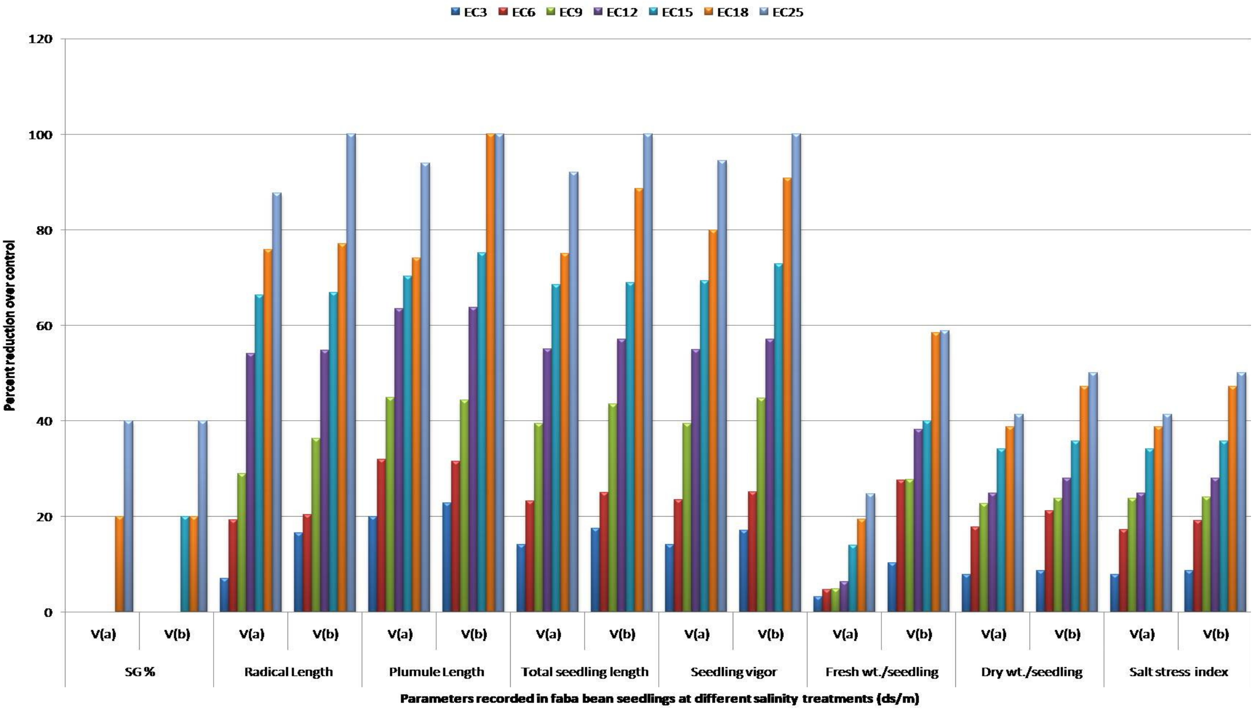

Fig. 5: Percent reduction in different characteristics of faba bean varieties [V(a) and V(b)] measured under different salinity treatments [EC0; Control, EC3, EC6, EC9, EC12, EC15, EC18 and EC25 (dS/m)].

Salinity stress index (Tsegay & Gebreslassie, 2014)

Salinity stress index (SSI %) is an important characteristic when selecting a variety for salinity tolerance. Salinity treatment causes reduction in this index. Higher valve of SSI (%) due to less reduction in dry weight can be used to assess salt tolerance in investigated genotypes. In this study, reduction in SSI ranged from 7.92 % to 41.26 % in V(a) and from 8.66 % to 50 % in V(b) from lower to higher salinity over control (Figure 5). The results revealed that the faba bean variety V(a) maintained higher value of SSI (%) due to less reduction in dry weight over control which showed its more resistance towards salt stress (Table 1). However, the second variety of faba bean V(b) showed greater decline in SSI even under moderate salinity level leading to its greater sensitivity towards applied salinity (Figure 5).

Optimization of salinity stress level

Both faba bean genotypes showed significant variations in all the measured characteristics at different salinity levels. But more comparable results were observed at EC15 (ds/m) in this study where a percent reduction of 50-70 % was measured in all seedling growth characteristics. At high salinity (EC 25 ds/m), greater reduction was observed (Figure 5). Therefore, present study suggests that EC15 can be taken as a optimum salinity level for further screening of faba bean for vegetative and reproductive stages so that they can adapt, survive and produce significant grain yield. This may help in selection of salt tolerant crop genotypes for saline soil.

Discussion

Seed germination is the primary stage of plant development. Salinity treatments in this study showed significant reduction or inhibition of seed germination in investigated local varieties of faba bean having variable response. Salinity delayed the seed germination more in faba bean variety V(b) than V(a). The results are in accordance with the earlier findings (Kumar, 2017; Sehrawat *et al*., 2014e, 2016; Shanko *et al*., 2017; Shu *et al*., 2017). Decrease or delay in seed germination under salinity stress may be either due to increased seed dormancy or due to insufficient water uptake as a result of ion toxicity (Na^+ and Cl^- ions). Less water uptake causes poor activation of hydrolytic enzymes resulted in decrease in seed germination under salinity stress (Jamil *et al*., 2006; Panda, 2001; Sehrawat *et al*., 2014e, 2016; Shanko *et al*., 2017). The potential of seeds to germinate under salinity may be interrelated with the tolerance at later growth stages of the crop (Sehrawat *et al*., 2016).

The seedling growth and biomass characteristics were also decreased with increased level of salinity and the results are in agreement with the earlier reports (Al-Mutawa, 2003; Haileselasie and Teferii, 2012; Sehrawat *et al*., 2014e, 2016; Tsegay and Gebreslassie, 2014). Radical length was found to be less affected due to salinity as compared with the plumule length even at high salinity. This showed more adjustment of the roots to establish the crop plants under salinity stress. Greater reduction of plumule growth may be due to less absorption of water and poor supply of nutrients during germination. Salinity induced osmotic stress and ion toxicity may be responsible for this behavior. But V(a) showed less reduction in seedling growth and biomass than V(b) in this study which suggest its more resistance towards salinity. Salinity interferes with the plant growth and development processes such as cell division, cell enlargement, prevent nutrient uptake resulted in inhibition of growth. Decrease in seedling growth and biomass may be due to altered metabolic activities and reduced transition of nutrients from cotyledons towards plumule and radical under salinity (Al-Mutawa, 2003; Sehrawat *et al*., 2014e, 2016; Waheed *et al*., 2006).

As a result of decrease in seedling length and dry weight, seedling vigor and salinity stress index also decreased with increase in salinity. These results are in accordance with the earlier findings (Hosseini *et al*., 2003; Jamil *et al*., 2006; Sehrawat *et al*., 2014e, 2016; Tsegay and Gebreslassie, 2014). But the variety V(a) tried to maintain less reduction in seed germination, seedling growth and biomass than V(b). These results demonstrated salt resistance nature of V(a) in comparison to V(b). Salinity tolerance during germination and seedling establishment in crop plants helps in its survival during vegetative and reproductive stages under saline environment that may produce stable yield at maturity. Faba bean variety V(a) tried to maintain less reduction in seed germination and seedling growth characteristics (particularly, radical length, seedling vigor and salinity stress index) even at high salinity (EC25 ds/m) showing its greater ability towards salinity stress than the variety V(b) which showed reverse response in this study. Therefore, faba bean local or wild germplasm can be explored at large scale for salinity tolerance to identify salt resistant genotypes for breeding as well as for salinity affected area. Selection and development of more salt tolerant varieties of faba bean would help to improve its productivity on saline soil (Sehrawat and Yadav, 2020; Sehrawat *et al*., 2019; Sehrawat *et al*., 2020b; Tavakkoli *et al*., 2012).

Conclusions

Faba bean is an important pulse crop with high nutritive value along with medicinal, agricultural and industrial importance. Salt tolerance during seedling growth stage helps in better establishment of seedling under salinity. This may results in more adaptations and healthy plants development during later growth stages (vegetative growth and reproductive development) to produce significant yield under salinity. Screening of large number of plants in effective manner may help to identify and selection of crop specific salt tolerant lines/varieties. These can be used to; i) identify candidate genes/major gene(s)/loci governing salt tolerance, ii) study detailed mechanisms or pathways responsible for salt tolerance in legumes and iii) provide promising genetic material as donors of valuable features for the breeders to develop new cultivars with increased resilience towards salinity. Therefore, collection and evaluation of local genetic resources of faba bean is required for genetic improvement of salt sensitive legumes in future.

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