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Attenuation of the Effect of Salinity by *Ascophyllum nodosum* Extract on Growth Associated Traits of Mung Bean Cultivars [*Vigna Radiata* (L.) Wilczek]

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ABSTRACT: Mung bean [*Vigna radiata* (L.)] is grown for its protein-rich edible seeds. Its productivity in the last few decades is stagnant because of biotic and abiotic constraints. Among the abiotic factors, salinity is of paramount importance as it severely affect the productivity of sensitive crops like legumes. Salinity has a deleterious effect on the growth associated traits at an early stage of the seedling. Brown alga, *Ascophylum nodosum* extract (ANE) impart stress tolerance in sensitive crops. Hence, four genotypes of Mung bean were screened to assessthe effect of different concentrations of NaCl and *Ascophyllum nodosum* extract (ANE) individually and in combinations on early seed vigor parameters. The germination process was evaluated daily up to seven days and observations like Percent Germination (PG), Germination Rate (GR), Germination Capacity (GC) and Germination speed (GS), Vigour Index (VI), Coefficient of Velocity of Germination (CVG), Seed Vigour Index (SVI), Standard germination (StG), Membrane Stability Index (MSI), Hypocotyl length (HL), Root length (RL) and secondary root number were recorded. All cultivars behaved differently. MH 215 was better for GC, VI, SVI, HL, RL and secondary root and MH 421 was superior for GS, StG, MSI. However, per cent germination and GR were better in MH125 and MH 318. It was concluded that a low concentration of ANE individually or in combinations with low salt ameliorated the effect of NaCl on growth attributes in Mung bean.

Keywords: *Vigna radiata, Ascophyllum nodosum* extract, Salinity, Growth associated traits

Globally, agriculture productivity is inhibited by abiotic and biotic stresses, but abiotic stresses in particular [1] affect the spreading of plant species across different environmental zones [2]. Drought, high temperature and salinity are the major abiotic constraints. Legumes are very important food and feed crops, known for their health benefits [3]. It is grown principally for its protein-rich edible seeds that are used as human food. Its herbage is used as fodder and green manure [4]. India is the prevalent producer of pulses which contributes 35.7% to global production [5]. Under this situation, the widely accepted benefits of legumes in cropping systems are needed now more than ever [3-6]. Mung bean (*Vigna radiata* (L.) Wilczek, Fabaceae) is an important legume crop. It is cultivated on greater than 6 million ha in the warmer regions of the world. It is a short duration (65– 90 days) grain legume having wide adaptability and low input requirements [7]. Mung bean is generally known as a salt-sensitive crop, and reduction of its growth increases with increase in saline regimes [8]. Salt stress reduces seed germination, fresh and dry biomass, shoot and root lengths and yield attributes of mung bean [9-11]. The salt injury also leads to pronounced symptoms like enhanced chlorosis, necrosis and decreased content of chlorophyll and carotenoids in mung bean [12]. NaCl stress had a more deleterious effect on roots than shoots, with a sudden dip in root growth associated traits [10]. Seaweed extract has been used as a natural organic fertilizer in the agricultural system due to its effective and nutritious benefit to crops. It is a new generation natural organic fertilizer that promote germination of seeds, increase in yield and resistant ability of many crops [13]. Seaweed extracts have an advantage as they are free from any weeds and pathogenic fungi. Liquid extracts of

Phaeophyta are being sold as biofertilizers and biostimulants in different brand names. The brown algae, *Ascophyllum nodosum,* is the most commonly used seaweed in commercial extracts and suspensions. *Ascophyllum nodosum* is thoroughly studied for its use in agriculture because of its extraordinary capacity to crop plants [14]. The *A. nodosum* contains organic acids, macronutrients, micronutrients and various hormones like cytokinins, auxin, gibberellins, betaines, mannitol and proteins that are important for the plant growth [15]. Application of *A. nodosum* extract (ANE) has also been shown to impart stress tolerance in sensitive crop plants. Studies on Citrus, Grapes, Bermuda grass and Kentucky bluegrass have demonstrated that ANE improved abiotic stress tolerance [16-17], create resistance to environmental stress [18] and enhance antioxidant properties, increase nutrient uptake from soil [19]. The tolerance during germination stage is critical for field emergence and early seedling vigour. Therefore, the objective of this study was to evaluate the four mung bean genotypes for sensitivity to salinity during the early germination stage. The different concentration of NaCl and ANE individually and with various combinations were applied for alleviating the effect of NaCl on germination and on various early seed vigor parameters in laboratory conditions.

MATERIALS AND METHODS

Plant Materials, *Ascophyllum nodosum* Extracts (ANE) and Treatment Combinations

Mung bean (*Vigna radiata* L.) genotypes *viz.,* MH 215, MH 421, MH 318, MH 125 were procured from the Pulses Section, Department of Genetics and Plant Breeding, CCS Haryana Agriculture University, Hisar, Haryana (India). Seaweed, *Ascophyllum nodosum* (Trade name: Biovita) extract (ANE) was purchased from Rajasthan, India. Five concentrations (0.00%, 0.01%, 0.05%, 0.10% and 0.50% v/v) including control of ANE were prepared by diluting the *Ascophyllum nodosum* with sterilized distilled water. Different concentrations of NaCl *viz.,* 0 mM, 25 mM, 50 mM, 75 mM, 100 mM (w/v) were prepared in distilled water. The salt (NaCl) and ANE were used individually as well in combinations (Table 1). All the four genotypes were screened for assessing the effect of different concentrations of NaCl and *Ascophyllum nodosum* extract (ANE) individually and in various combinations for germination and various early seedling vigour parameters.

Growth parameters

Mung bean seeds of four above mentioned genotypes which are uniform, homogeneous and identical in size and colour, and free from wrinkles were selected and surface sterilized with 0.1% $HgCl_2$ for 5 minutes. The seeds were washed thoroughly (2-3 times) with sterilized distilled water. Healthy seeds of all four genotypes were then soaked in 20 ml of distilled water for 12 hours. Twelve hydrated seeds of each genotype were placed in triplicate on a filter paper in 9 cm petri plates containing 10 ml of each combination of salt and ANE (Table 1). The germination process was evaluated daily up to seven days, and observations were recorded for each treatment. Seeds were considered to have germinated when radical had emerged and elongated by at least 2 mm. One or 2 ml of respective treatment solutions, depending on the requirement, was applied. Germination percentage was recorded on three days after incubation. Modified and standard protocols were followed for this observation, and a seed able to protrude radicle was considered germinated [20]. Observations like daily radicle length, secondary roots and hypocotyl length were also recorded up to seven days.

Table 1. Experimental design of different treatments combinations

ANE (%) &	NaCl (mM) & Code				
Code	0	25	50	75	100
0.00 (A0)	0.00+0 (T1)	0.00+25 (T2)	0.00+50 (T3)	0.00+75 (T4)	0.00+100 (T5)
0.01 (A1)	0.01+0 (T6)	0.01+25 (T7)	0.01+50 (T8)	0.01+75 (T9)	0.01+100 (T10)
0.05 (A2)	0.05+0 (T11)	0.05+25 (T12)	0.05+50 (T13)	0.05+75 (T14)	0.05+100 (T15)
0.10 (A3)	0.10+0 (T16)	0.10+25 (T17)	0.10+50 (T18)	0.10+75 (T19)	0.10+100 (T20)
0.50 (A4)	0.50+0 (T21)	0.50+25 (T22)	0.50+50 (T23)	0.50+75 (T24)	0.50+100 (T25)

Seed vigour parameters were calculated as suggested by [21] as below:

Germination rate (GR) = (100/n) (N3/3 + N5/5)

Germination speed (GS) = (100/n) (N1/1 + N2/2 + N3/3 + N4/4 + N5/5 + N6/6 + N7/7)

Germination capacity (GC) = (100/n) (G3)

Standard germination (StG) = Final count (G/100)

Coefficient of Velocity of Germination (CVG) = 100 (N1 + N2 +…+ N7)/ (N1 × 1 + N2 × 2 + N3 × 3 + N4 × 4 + N5 × 5 + N6 × 6 + N7 × 7)

Vigor Index (VI) = (1/2) (100/n) (H4 + R4)

Where n is number of germinated seeds; N1, N2… N7 indicate number of normal seedlings at one, two … seven days after germination and 1, 2…7 indicate one, two … seven days after the start of germination test; G3 is number of seedlings with more than 2 cm radicle length three days after germination; H4 is number of seedlings with hypocotyl length more than average four days after germination; R4 is number of seedlings with root plus hypocotyl length more than average four days after germination.

The standard protocol of Abdual-Baki and Anderson [22] was used for calculating the Seedling Vigor Index (SVI) as:

SVI-I = Germination (%) × Seedling length (cm)

Membrane stability index (MSI)

Standard protocol of Sairam *et al*. [23] was followed for determination of Membrane stability index (MSI) which was recorded by observing the electrical conductivity of leaf leachates in double distilled water at 40^0C and 100^0C. Leaf samples (0.1 g) from all combinations were cut into discs of uniform size and the put in test tubes containing 10 ml of double distilled water in duplicate. One set was kept at 40^0C for 30 min and another set at 100^0C for 15 min and then their respective electric conductivity C1 and C2 were measured by conductivity meter. The MSI was calculated by the following formula:

$$\text{Membrane stability index} = \left[1 - \frac{C1}{C2}\right] \times 100$$

RESULTS AND DISCUSSION

In plants, the most critical stage during seedling growth is the seed germination that determines effective crop establishment and production. The combinations of *Ascophyllum nodosum* with salinity had a beneficial effect on the germination of *Vigna radiata*. Comparison between the respective treatments of salinity with and without *Ascophyllum nodosum* extract showed that germination percentage increased with increasing *Ascophyllum nodosum* concentrations but, at higher concentration germination decreased. *Ascophyllum nodosum* was effective in alleviating the inhibitory effect of salt stress during seed germination.

Germination Percentage

Germination percentage decreased with increase of salinity in all the tested genotypes. The average highest germination percent was achieved in MH 125 (98.99%) which was at par with MH 318, while lowest was found in MH 421 (75.64%) significantly. The interaction between all the concentrations of NaCl, results showed that lowest germination percent was achieved with 100 mM NaCl (80.8%)(Figure 1a and 1b). Similarly it was observed that increased salinity levels during seed germination significantly reduced the germination and seedling performance [24- 25]. The inhibition of seed germination induced by salt stress was related to the suppression of ethylene production during imbibitions. Salt stress decreased germination by preventing the germination processes due to the accumulation of a high concentration of Na^+ and Cl^- ions, which might be toxic to the embryo or the developing seedlings [25-26]. Germination percentage was reduced in wheat seeds in response to increasing concentration of NaCl [27]. Higher concentrations of ANE (0.10% and 0.50%) decreased the germination percentage as compared to the lower concentrations (0.01% and 0.05%) of ANE. MH 318 behaved better for seed germination as compare to other genotypes at 0.50% ANE. At the lower concentration (0.01% and 0.05%) of ANE germination increased with all combination. Seeds of tomato treated with *U. lactuca* and *P. gymnospora* at 0.2 % concentration, increased the germination percentage and at higher concentration germination percentage decreases. [28]. *Vigna mungo* seeds when were soaked in a lower concentration of seaweed extract showed a higher rate of seed germination while high concentration reduced the seed germination [29]. The increased germination percentage at low concentrations could be due to the presence of growth-promoting substances such as indole acetic acid, indole-3-butyric acid, gibberellins, cytokinins, micronutrients, vitamins, and amino acids [30]. The effect

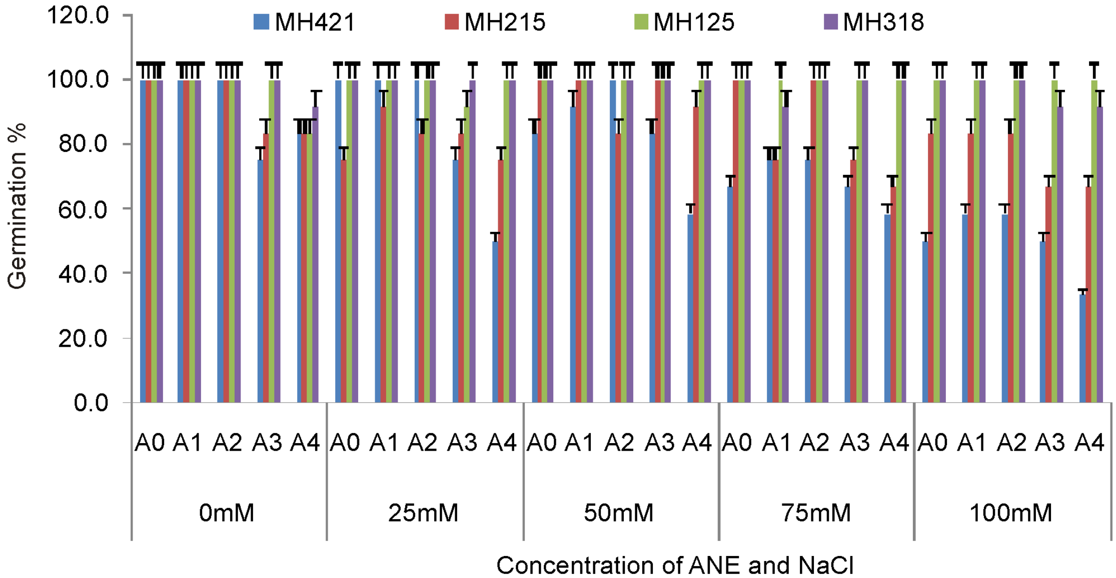

Figure 1a. Germination percentage of four mung bean genotypes under different levels of NaCl and ANE alone and in combinations

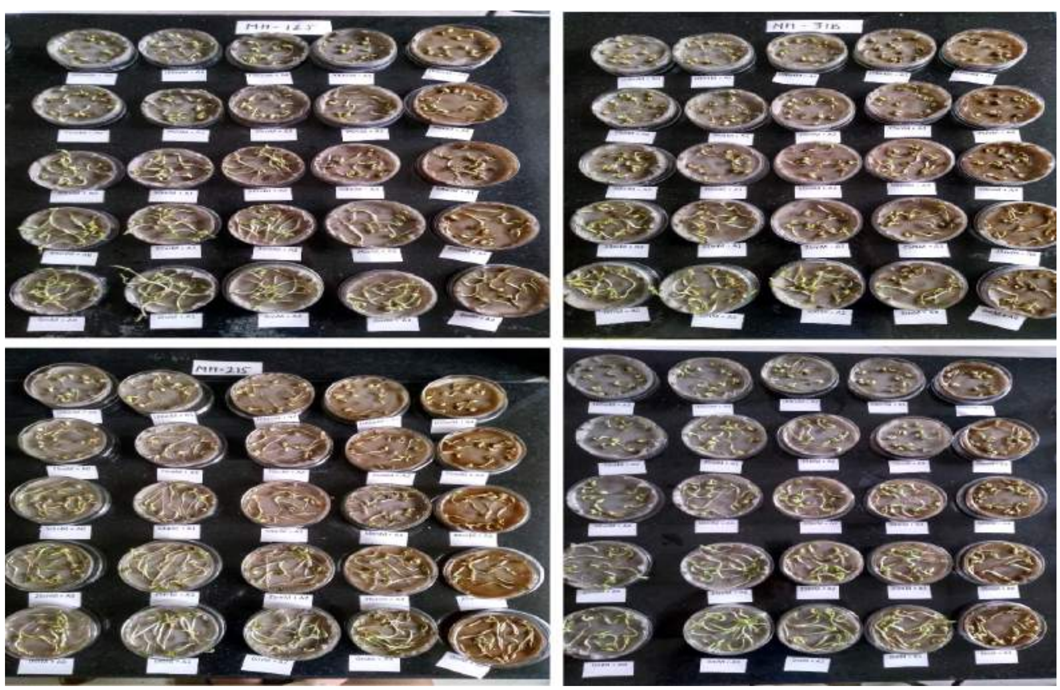

Figure 1b. Germination of four mung bean genotypes under different levels of NaCl and ANE alone and in combinations

of seaweed liquid extract (SLE) of *Sargassum wightii* on germination, of *Triticum aestivum* var. Pusa Gold and found that application of 20% SLE showed a 63.38% increase in the number of lateral root and enhanced the percentage of seed germination as compared to control [31].

Germination Rate (GR)

Germination rate (GR) was higher in MH 421 at all the tested concentration of salt as compare to other genotypes. It was indicated that MH 421 have better tolerance to is salinity among the four tested genotypes. Out of all the salinity concentrations, GR was found

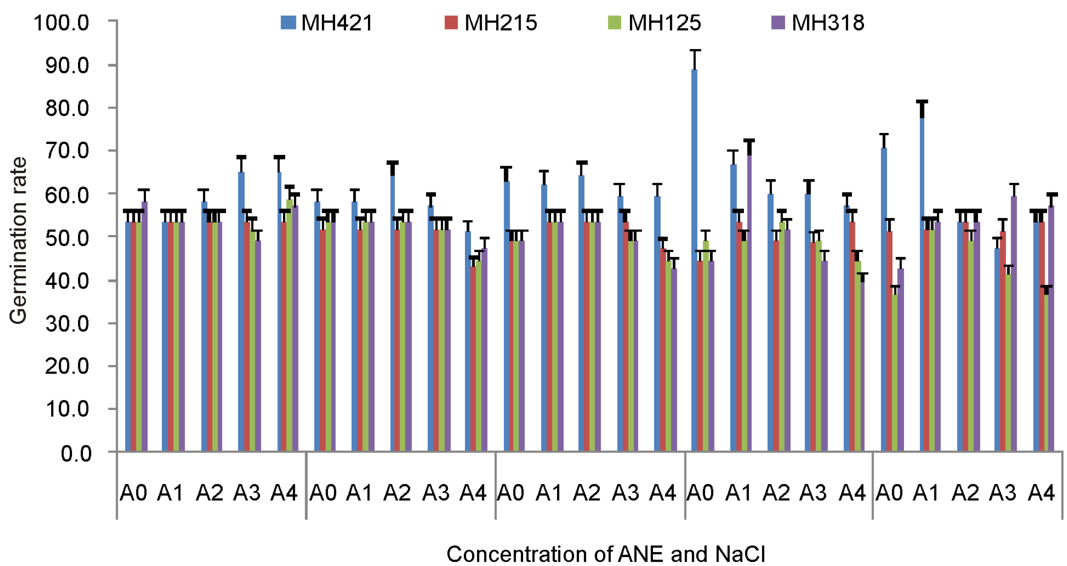

Figure 2. Germination rate of four mung bean genotypes under different levels of NaCl and ANE alone and in combination

highest in 75mM NaCl concentration with MH 421 (61.07) followed by MH318 (51.64) and MH215 (51.30) (Figure 2). Higher concentration of salt and ANE decreased the germination rate in all four genotypes. Foolad and Lin [32] observed that high salt concentration reduced the germination rate in tomato Though combination of the lower concentrations of ANE 0.01% and 0.05% with all the tested concentration salts were better for increasing the rate of germination. At these two concentrations the rate of germination was better than that of control. Liquid seaweed extracts of *U. lactuca* and *P. gymnospora* at concentration of 0.2%, increased the germination rate but at higher concentration germination rate was found to be decreases [28]. Salinity may affect the germination of seeds either by creating an osmotic potential external to the seed which prevents water uptake, or through the toxic effects of sodium and chloride ions on the germinating seed. During germination under saline conditions, high osmotic pressure of saline water is created due to capillary rise leading to more salts density at seed depth than at lower soil profile, which reduces time and rate of germination [33].

Germination Capacity (GC)

Germination capacity (GC) was greatly affected by salt in all the genotypes, GC decreased with increasing salt concentration (Figure 3).

GC greatly reduced and become zero at 75mM and 100mM NaCl in all genotypes except MH 215. At 75mM NaCl, GC of MH 215 is 50, it means MH 215 has better tolerance to salt stress. Higher concentration of ANE decreased the GC in all genotypes. At lower concentrations i.e., 0.01% and 0.05%, of ANE, GC increased at all salinity level. Overall MH 215 was the better cultivar at all the tested concentrations.

Germination Speed (GS)

Germination speed was fount maximum in MH 421 (281.67) followed by MH 318 ((254.12). GS decreased significantly with increasing of salt except MH421. Low concentration of ANE (0.01%) in combination with all the tested concentrations of NaCl was found to be better for GS. Untreated plants behaved better than treated plants (Figure 4). It is well established that salt stress has negative correlation with seed germination of *Vicia faba* [34]. Higher level of salt stress inhibits the germination of seeds while lower level of salinity induces a state of dormancy [35]. Increasing salinity leads to a reduction and/or delay in germination of plants and death of seeds before germination [36].

Vigour Index (VI)

For the vigor index, MH 215 was the better genotype when grown under different salt concentrations. Overall, 75mM concentration of salt was better for VI (56.93) flowed by 50 mM (53.07) for all the tested genotypes of Mung bean (Figure 5).

It was also observed that 0.10% and 0.50% ANE showed highest VI at all the tested concentration of salt. At higher concentration (0.50%) of ANE, VI was highest at 100mM

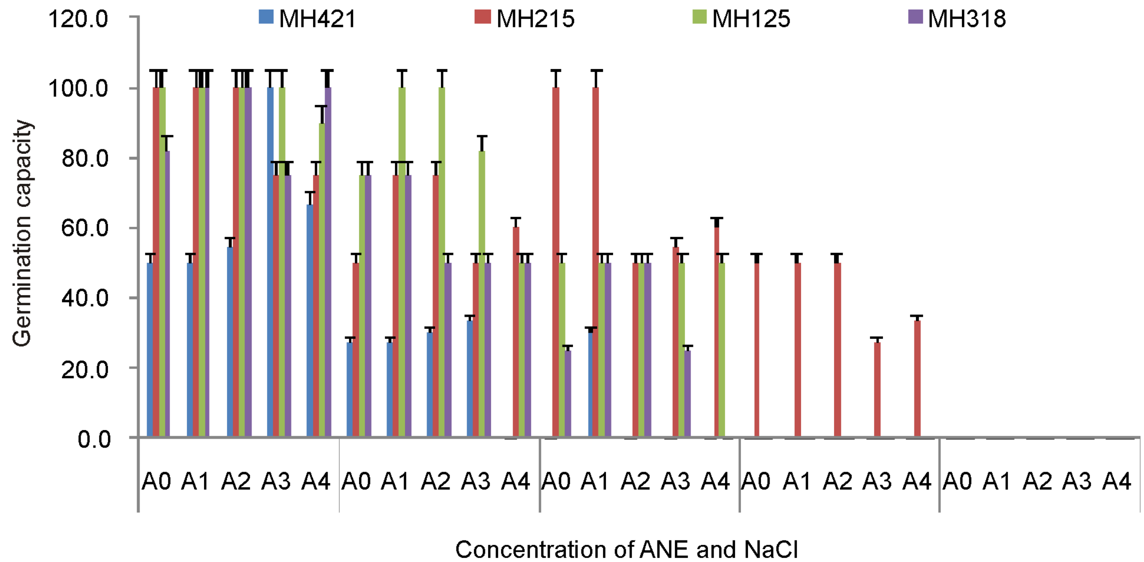

Figure 3. Germination capacity of four mung bean genotypes under different levels of NaCl and ANE alone and in combinations

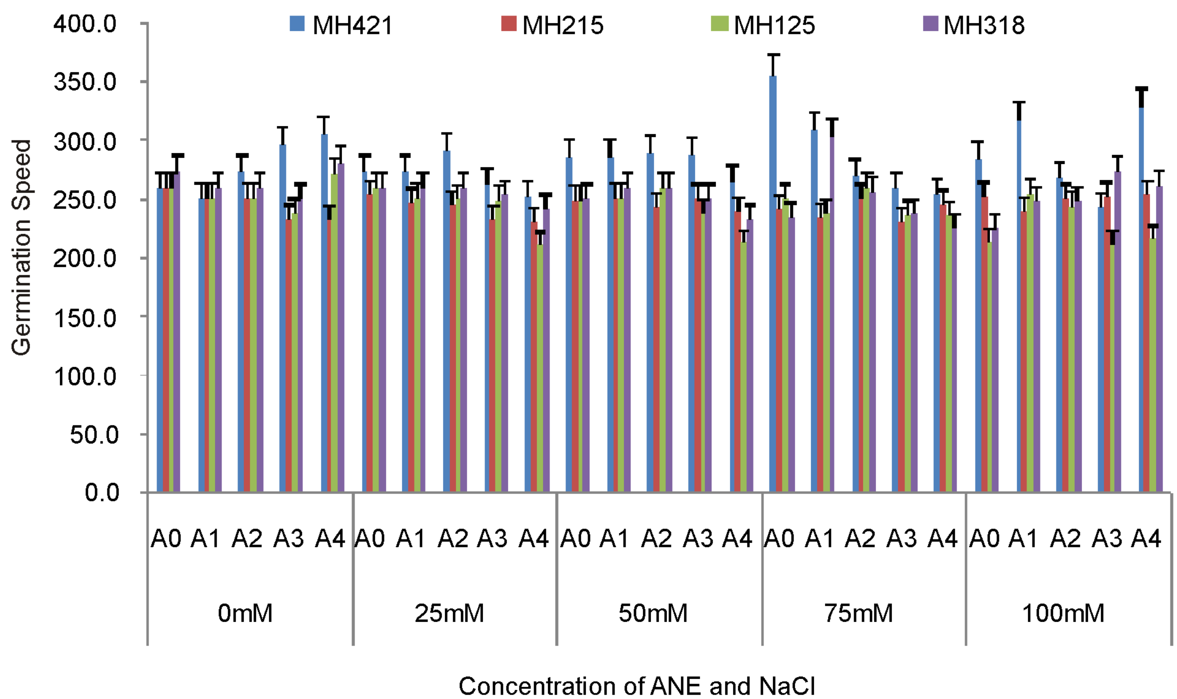

Figure 4. Germination speed of four mung bean genotypes under different levels of NaCl and ANE alone and in combinations Germination speed

salt stress in MH215 (67.82) which showed that MH 215 had greater vigour index in comparison to all other genotypes.

Coefficient of Velocity of Germination (CVG)

Coefficient of the velocity of germination was almost similar for all the four genotypes at all the tested concentrations of salt which was non-significant.100 mM salt showed more CVG for all the four genotypes (Figure 6). It was also observed that 0.50% of ANE showed maximum CVG with all combinations of salt. All seedling vigour parameters like germination rate(GR), germination speed(GS), germination capacity(GC), standard germination(StG), and coefficient of velocity of

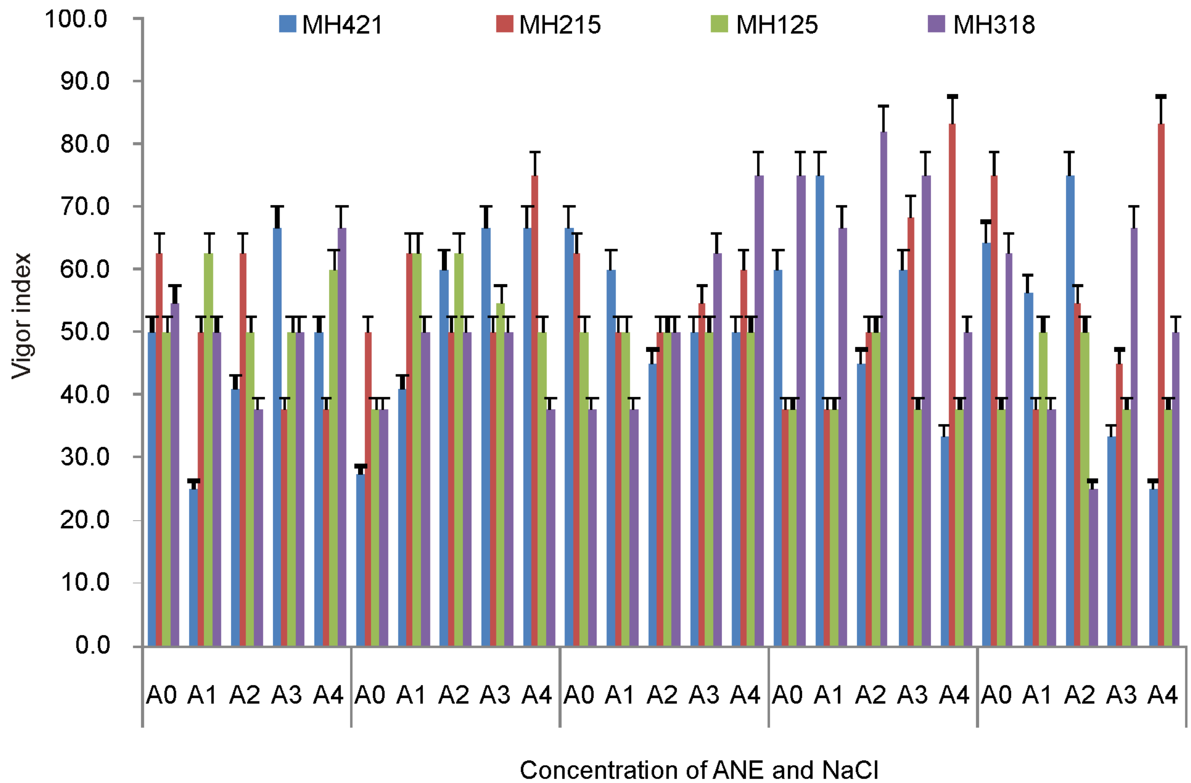

Figure 5. Vigor index of four mung bean genotypes under different levels of NaCl and ANE alone and in combinations

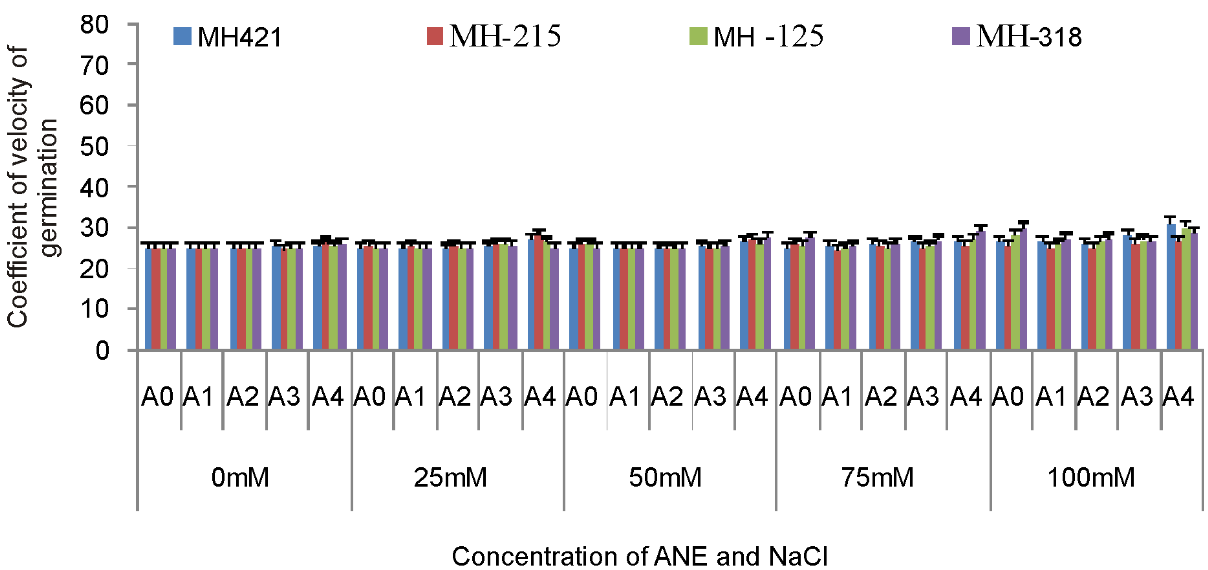

Figure 6. Coefficient of velocity of germination of four mung bean genotypes under different levels of NaCl and ANE alone and in combinations

germination(CVG) were adversely affected with increasing of salinity levels [37]. According to Rehman et al.[38] toxicity of salinity is associated with the decrease of potassium ion content in the seed during germination, which reduced metabolic function and eventually reduced the germination and seedling growth. Osmotic and toxic effects occurs due to declining solute and uptake and/or accumulation of some ions in the seed[39].

Seed Vigor Index (SVI-I)

Seed vigour index was found positively significant and it was observed maximum in MH125(1176.7) (from rest of the genotypes. Overall, SVI-I was found greater at 0mM concentration of salt (1010.0) in MH 215 genotype as compared to rest of the NaCl concentrations and genotypes (Figure 7). It was observed that 0.01% and 0.05% of ANE with all combinations of salt showed better SVI-I. A study was also conducted earlier [40] to determine the influence of seaweed saps [*Gracilaria edulis* (G sap) and *Kappaphycus alvarezii* (K sap) on germination, growth and Seedling Vigor Index (SVI) in maize. Liquid seaweed extracts of *U. lactuca* and *P. gymnospora* at 0.2%, showed higher seed vigour index [28].

Standard Germination (StG)

At all the concentrations of ANE, MH 215) had the highest standard germination followed by MH 318 and MH 215 . The concentration of ANE like 0.01% and 0.05% were found to be better (100.0) for all the four genotypes. 0.01% and 0.05% ANE with all the concentrations of salt showed more StG (Figure 8). The genotype MH 215 was found to be superior at 100mM of NaCl with all combinations of ANE. Whole seaweed or processed or purified concentrations of seaweed have been used in agriculture to improve salt stress [41]. Germination from both treated and non-treated seeds with *Sargassum vulgare* decreased significantly with increasing salt stress. However, this reduction in total germination was significantly higher for non-treated seeds, compared to treated ones. Seeds treated with liquid seaweed extracts of *U. lactuca and P. gymnospora* at lower concentrations (0.2 %) showed enhanced germination i. e. better response in germination rate associated with lower mean germination time, high germination index and germination energy, and consequently greater seedling vigor and greater plumule and radicle length [28].

Membrane Stability Index (MSI)

Membrane stability index was found highest in MH421 (50.79) than other genotypes at all the concentrations of salt stress (Figure 9). In case of MH421 MSI decreased significantly with increasing of salt. MSI of MH 125 and

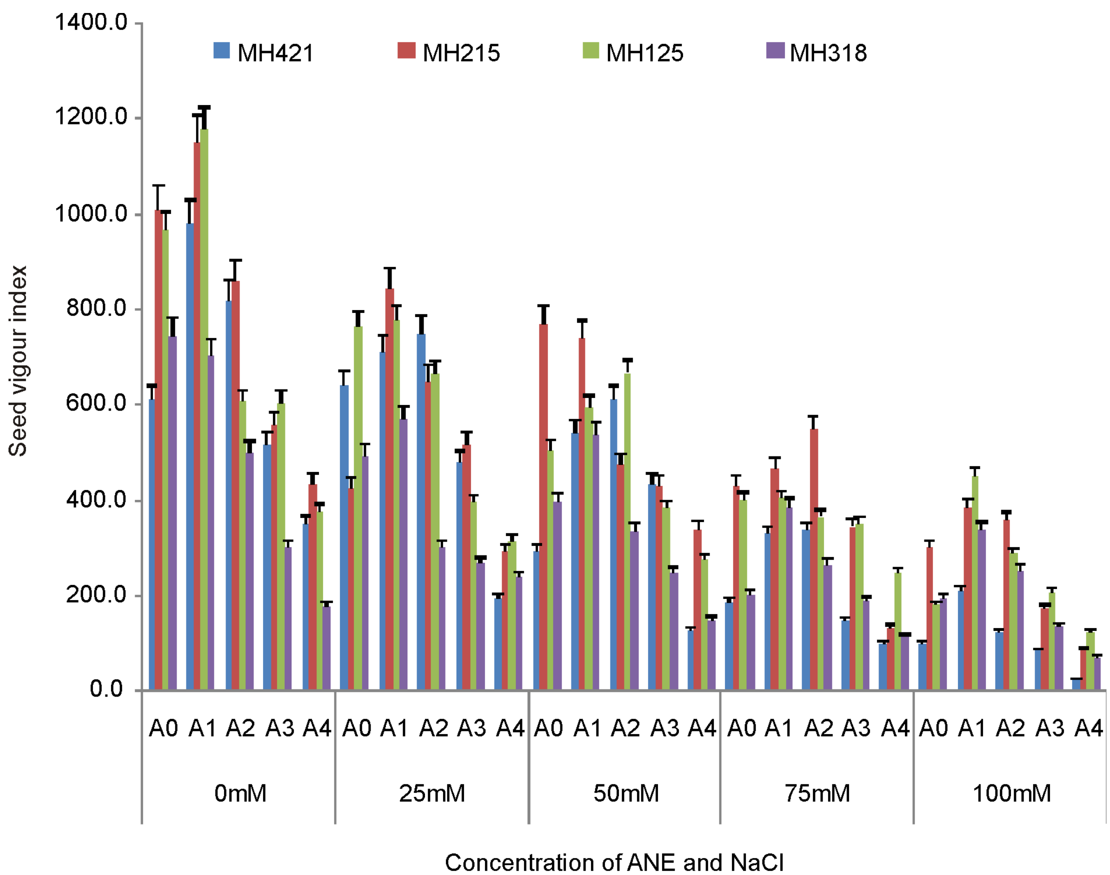

Figure 7. Seed vigor index (SVI-I) of four mung bean genotypes under different levels of NaCl and ANE alone and in combinations

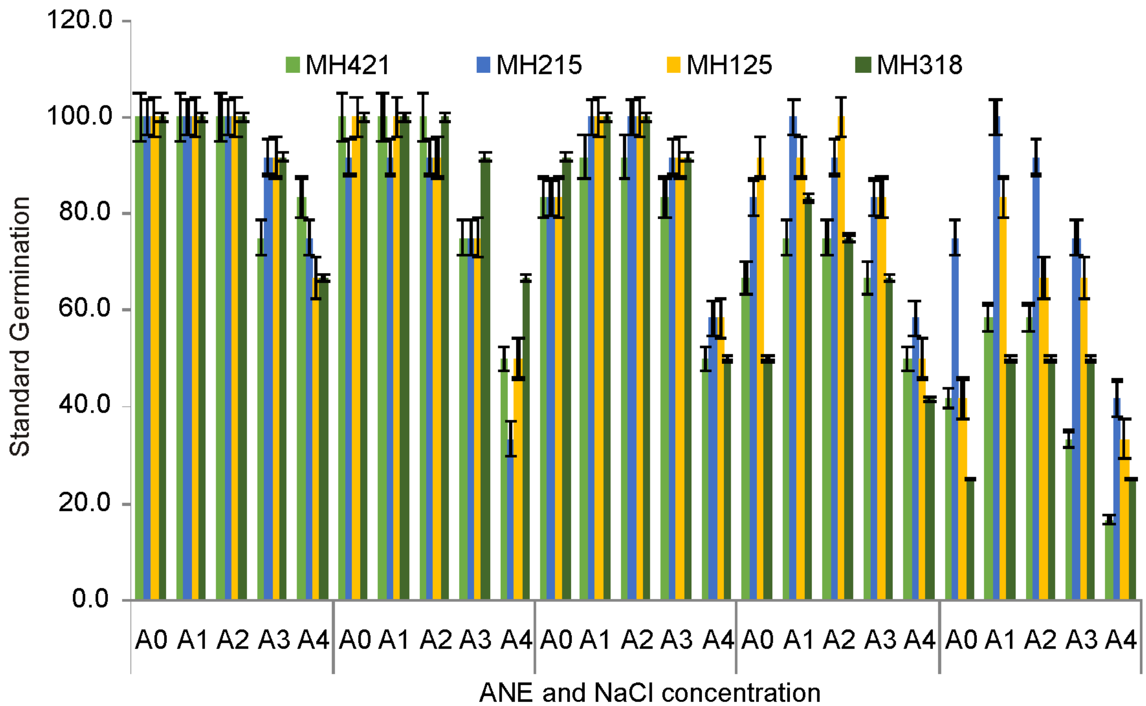

Figure 8. Standard germination of four mung bean genotypes under different levels of NaCl and ANE alone and in combinations

MH 318 increased at lower concentration (0.05%) of ANE as compare to control. Overall, results showed that higher concentration of ANE was deleterious for MSI with all the tested genotypes. In pea genotypes Membrane stability index (MSI) decreased under salt stress, Since membrane damage increased with increase in salt stress so it can be considered as very important tool for evaluation of salt tolerant crop[42]. It was reported in *Triticum aestivum* genotypes that MSI progressively decline due to increase in electrolyte leakage at different levels of salinity [43].

Hypocotyl Length

Hypocotyl length decreased with increasing of salt concentrations in all the four genotypes as compare to control. Hypocotyl length of MH215 was greater (2.9 cm) as compare to other genotypes at all salinity level (Figure 10). At lower concentration of ANE hypocotyl length increased in all the genotypes. MH125 and MH 215 had 7.3 and 6.9 cm, respectively, hypocotyl length at 0.01% concentration of ANE. Hypocotyl length increased at lower concentration of ANE with all the salt concentrations as compared to control for all the genotypes. Plant height of rice plants decreased progressively with increase in salinity levels [44]. The influence of salinity on the height has also been reported earlier [45, 46]. The brown seaweed, *Padina gymnospora* on tomato and mung bean plants increase germination percentage, radical and shoot length, and dry weight [47].

Root Length

Root length decreased at higher concentration of salt in all the genotypes significantly. Average root length of MH 215 was higher (4.6 cm) than the other tested genotypes at higher concentration of salinity (Figure 11).

Lower concentration (0.01%) of ANE increased the length of root in almost all genotypes. Root length of MH 215 was greater (2.6 cm) at all the concentrations of salt and ANE as compare to other genotypes. Overall, in this present study the root length was significantly increased with 50 mM NaCl in MH 215. The application of seaweed extract at different concentrations enhanced shoot length, root length and seedling vigour index, higher shoot length, root length and vigour index was noticed when seeds were soaked in 15% G sap or 15% K sap. The extract of seaweed *Ascophyllum nodosum* (Trade name: Biozyme) increases growth and yield parameters of soybean [48].

Secondary Roots

Secondary roots decreased with increasing of salt stress in all the genotypes (Figure 12). MH215 had more

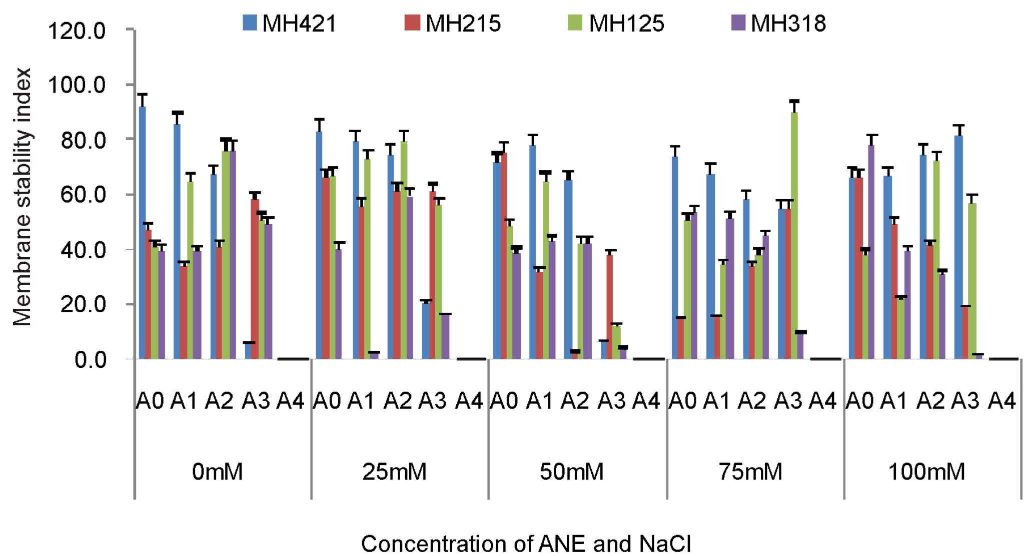

Figure 9. Membrane stability index of four mung bean genotypes under different levels of NaCl and ANE alone and in combinations

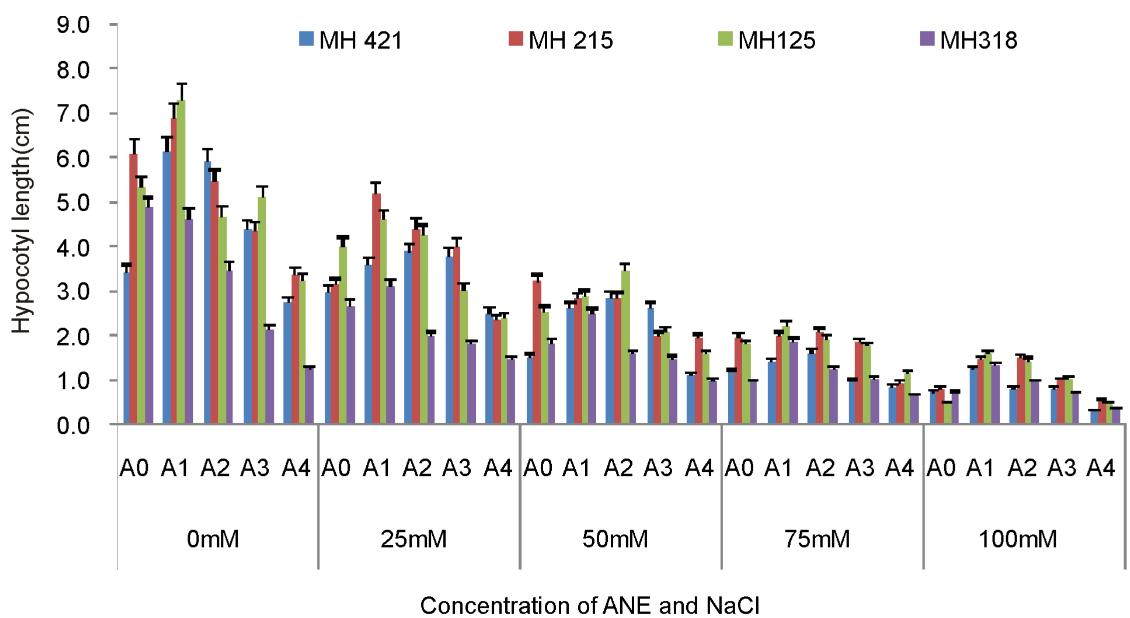

Figure 10. Hypocotyl length of four mung bean genotypes under different levels of NaCl and ANE alone and in combinations

secondary roots as compared to other genotypes. There was more number of secondary roots at low concentration of ANE with all salinity level in all genotypes. MH215 was the better for the origin of secondary root as compared to rest of the cultivars. The number of lateral roots in *Vigna sinensis* was observed to be highest at a 20% concentration, and the minimum number of lateral roots was recorded for a 100% concentration of *S. wightii extract* [49].

Salinity adversely affects the plant growth at all stages, particularly at seedling and reproductive stages, which dramatically reduced the yield [50]. ANE is responsible for inducing improved plant growth and enhance resistance to abiotic and biotic stresses [51]. Betaines protect against osmotic, drought, frost, high salinity and hightemperature stresses. It is also suggested that betaines may work as a nitrogen source when provided in low concentrations [52].

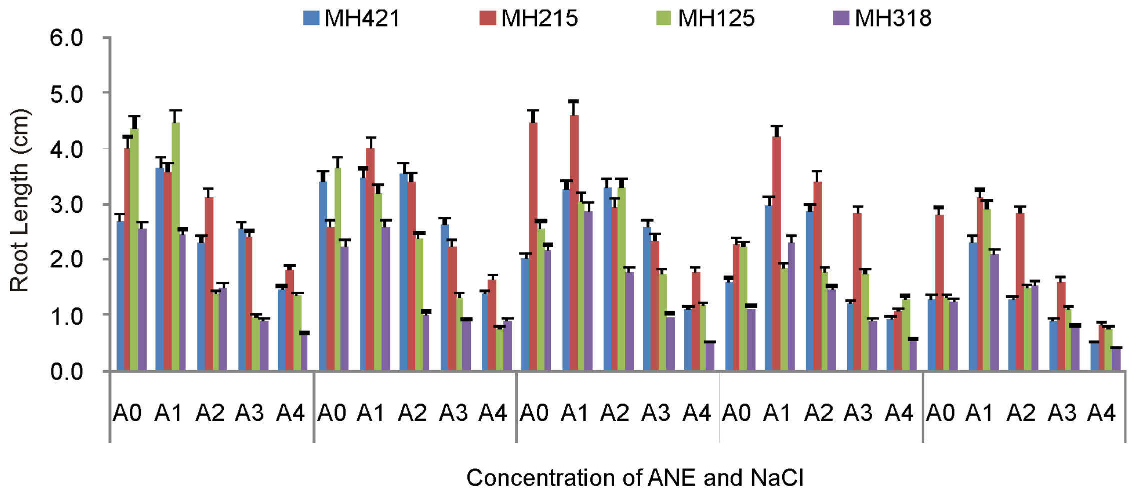

Figure 11. Root length of four mung bean genotypes under different levels of NaCl and ANE alone and in combinations

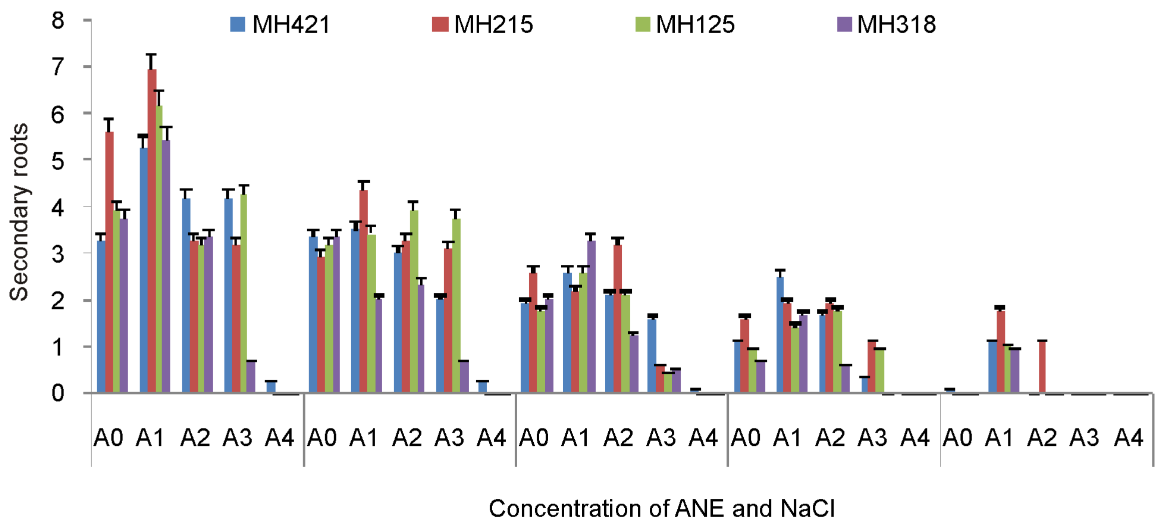

Figure 12. Secondary roots of four mung bean genotypes under different levels of NaCl and ANE alone and in combinations

It is concluded that the *Ascophyllum nodosum* extract promote growth, thereby, acts as a soil fertilizer. Its lower concentrations (0.01% and 0.05%) were found to be the best for overall growth as well as for mitigating the effect of salt in *Vigna radiata.*

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Conflict of Interest: The authors declare that they have no conflict of interest.

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