



Short Communication

Screening at early growth stage for identification of genotypes and physiological traits for salinity tolerance in grain amaranth genotypes

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Abstract

A prominent issue in the world's arid and semi-arid regions is salinity stress. It severely reduces seed germination and plant population, which results in a decreased yield. The present investigation was performed for identification of salt tolerant genotypes and traits conferring salinity tolerance at seedling stage. Ten genotypes of grain amaranthus species were sown at electrical conductivity of 0 dS/m (control), 5 dS/m (moderate stress) and 10 dS/m (high salinity stress). When exposed to salinity stress, the genotype GA-1 exhibited the highest germination percentage and speed of germination. Salt Tolerance Index (STI) at 5 dS/m electrical conductivity based on dry weight indicates that GA-1, GA-5, SUVARNA and IC-294449 had highest capacity to produce dry matter than other genotypes, with GA-1 maintaining maximum STI at higher salinity level. Root- shoot ratio and water status of seedling tissue were positively correlated at salinity level of 5 dS/m (0.1691) and 10 dS/m (0.4850). Membership function value (MFV) indicates the salinity tolerance of the genotypes i.e. higher MFV of a genotype indicates higher tolerance to salinity than other genotypes. The root shoot ratio was shown to be positively linked with MFV in the current study. The seedling vigour was positively correlated to MFV at 5 dS/m (0.9472) as well as at 10 dS/m (0.2172). The current study concludes that genotype GA-1 is relatively more tolerant, and traits like root-shoot ratio, seedling vigour, and seedling water content are important for selection of salinity tolerant genotype at seedling stage.

Key words : Root shoot ratio, Salinity, Speed of germination, Vigour, Water content

Grain amaranth, an important C₄ crop cultivated in tropical region, belongs to the family *Amaranthaceae* and genus *Amaranthus*. In India, three species of grain amaranth are cultivated: *Amaranthus hypochondriacus* L., *Amaranthus cruentus* L., and *Amaranthus caudatus* L.. Amaranthus grains are a high-energy diet with a high concentration of vitamins and minerals, as well

as a balanced composition of important amino acids (D'Amico et al., 2017). The crop is very efficient in water and nutrient utilization due to C₄ metabolic activity and thus can perform better under different abiotic stress exposure (Das, 2016). The crop, owing to its immense health benefits and wider adaptability to changing climatic patterns, offers a promising source for food and nutritional security.

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Salinity stress is one of the most severe abiotic stresses, affecting plant growth, physiological, biochemical and molecular traits. In India, according to Central Soil Salinity Research Institute, (Mandal et al., 2010) estimated that about 6.73 million hectares of agricultural land is salt impacted. Salinity is expected to impact half of the world's arable land by 2050 (Machado and Serralheiro, 2017). Soil salinity can be caused by an excess of chloride, sodium, and sulphate ions, which harm plants through osmotic stress and ionic toxicity (Chaves et al., 2009). Salt in the root zone alters plant metabolism and physiology, severely impacting germination, development, blooming, and fruiting, resulting in poorer yields (Safdar et al., 2019). Osmotic stress in the soil reduces the seed germination and ion toxicity reduces photosynthesis and ultimately yield of the plant (Mbarki et al., 2018).

The effect of salinity on plants varies greatly depending on species and genotype. Adoption of salt sensitive varieties for cultivation and non-availability of the tolerant cultivars results in more yield losses. Therefore, selection of salt tolerant varieties for cultivation under salinity is a must. The selection of salt resistant genotypes at the germination stage is critical since the seed's growth and development can be controlled by suitable management measures once germinated. Salinity tolerance evaluation through MFV comprises the salinity tolerance indices of all observed traits. Higher value of MFV of genotype suggests greater salt tolerance (Ding et al., 2018, Wu et al., 2019). The present investigation was envisaged to evaluate the traits conferring salinity tolerance in ten grain amaranth genotypes during early growth stages and to identify the best performing genotype.

Experimental materials: During *Rabi* season 2019-20, the experiment was carried out in the laboratory of the Department of Genetics and Plant Breeding, C. P. College of Agriculture S. D. Agricultural University, Sardarkrushinagar. The experimental material for the current study included ten genotypes

of *A. hypochondriacus* viz. GA-1, GA-2, GA-3, GA-4, GA-5, SUVARNA, *A. cruentus* viz. EC-198122, EC-198127, and *A. caudatus* viz. NIC 22553, IC294449 obtained from the Centre for Crop Improvement, S. D. Agricultural University, Sardarkrushinagar.

Prior to seed sowing, for 5 minutes, amaranth seeds were sterilized in a 5% sodium hypochlorite solution. Twenty seeds of each genotype in three replications were sown in sterilized, petri dishes (100mm × 15mm) containing Whatman filter paper no. 1. The filter papers were dampened with half strength Hoagland solution enriched with T₁: Control (double distilled water), T₂: 5 dSm⁻¹ T₃: 10 dSm⁻¹. The electrical conductivity of 5 and 10 dSm⁻¹ of salt solution was prepared using different salts (Table-1) (Arora et al., 2018). The petri plates were covered to prevent the decrease in water content from the petri dish so that the osmotic and ionic conditions do not change. Data pertaining to germination and related parameters were recorded.

Table-1 : Chemicals applied per litre of distilled water to provide required electrical conductivity.

| Name of the chemical | Chemical dissolved per litre (g/L) | |
|--------------------------------------|------------------------------------|---------|
| | 5 dS/m | 10 dS/m |
| NaCl | 0.761 | 1.156 |
| Na ₂ SO ₄ | 0.462 | 0.702 |
| CaCl ₂ ·2H ₂ O | 1.490 | 3.400 |
| MgSO ₄ ·7H ₂ O | 1.252 | 2.2889 |

Germination assessment

GERMINATION PERCENTAGE (%): The germination percentage was calculated using (Association of Official Seed Analysis, 1983). The number of germinated seeds was counted every 24 hours after seeding for six days, resulting in the final germination count.

Germination percentage = (No. of seed germinated / Total No. of seed sown) × 100

Speed of germination (day⁻¹)—Speed of germination was estimated using the method published by Maguire (1962)

Speed of germination = $N_1/D_1 + N_2/D_2 + \dots + N_n/D_n$
Where, N = Number of normal seedlings

D = Day of count

Seedling growth assessment

Root length (cm): After 6 DAS, the maximum root length was measured using a scale from the base of the shoot to the longest root tip. The data were collected for 5 seedlings per replication, and the average was reported in centimeters.

Shoot length (cm): After 6 DAS, the maximum shoot length was measured using a scale from the root and shoot junction to the tip of the shoot. The data were collected for 5 seedlings per replication, and the average was reported in centimeters.

Seedling dry weight: The dry weight of the seedling was determined after it had been oven dried at 70 °C.

Physiological indices for salinity tolerance

Water content of seedling was measured by using the following formulae:

$$\text{Water content} = (\text{FW} - \text{DW}) / \text{FW} \times 100$$

Where: FW is fresh weight of seedling and DW is dry weight of the seedling

Root shoot ratio of the genotypes under different level of salinity was calculated by dividing root length with shoot length.

Seed vigour index (dry weight basis)- Seed vigour index was calculated by the method described by Abdul Baki and Anderson 1973.

SVI = Germination percentage × Seedling dry weight

Stress tolerance evaluation

Stress tolerance index: Stress tolerance index (STI) was calculated on the basis of biomass/ yield per plant at harvest according to formula of (Fernandez, 1992).

$$\text{STI} = Y_p \times Y_s \div \text{mean} Y_p^2$$

The seedling dry weights of genotypes assessed under saline (stress) and non-saline (non-stress) conditions are denoted by Y_s and Y_p .

Salt tolerance evaluation The salt tolerance of *Amaranthus* species was assessed using the fuzzy comprehensive evaluation method and the membership function value (MFV) (Chen et al., 2012). The following equation was used to compute the MFV of salt tolerance:

$$X_i = [(X - X_{\min}) \div (X_{\max} - X_{\min})] \times 100\%$$

Where X_i represents the MFV of STI in a genotype, X represents the actual measured value of STI in a genotype, and X_{\max} and X_{\min} represent the maximum and minimum values recorded across all genotypes (Ding et al., 2018). The salt tolerance of the genotypes was evaluated based on the average value of the MFVs of each observed trait.

Statistical analysis

To get a statistically valid result, the data were statistically analyzed using OPSTAT software, which is available online at CCS Agricultural University's HISAR website (<http://hau.ernet.in>) for analysis of variance (ANOVA) using a two-factorial completely randomized design.

Genotypic variability in germination per cent under salinity stress

In the present investigation significant genotypic variability existed for germination percent in different genotypes of grain Amaranth species. The average germination percentage was reduced by 6.48 and 11.0%, respectively, when the salinity level was raised with electrical conductivity of 5 dS/m and 10 dS/m, respectively. This decrease in germination percentage can be attributed to lower water potential under high salinity (Munns and Tester 2008). The genotypes GA-1 and EC-198127 had the highest value at 5 dS/m (T2) (83.33) while EC-198122 had the lowest value (73.33). GA-1 reported the highest germination percentage (80%) at 10 dS/m (T3). Decreasing order of genotypes pertaining to germination percentage at 10 dS/m was GA-1 > GA-2 > GA-4 = GA-5 = EC-198127 > SUVARNA = IC-294449 > GA-3 = NIC-22553 > EC-198122 (Fig. 1). In the present investigation, increase in salinity level decreased the germination percentage, however, the genotype GA-1

maintained the maximum germination percentage under moderate (5 dS/m) and high salinity (10 dS/m) stress conditions. There has been evidence of a decrease in crop germination percentage under saline stress, including Amaranth species (Agapit et al., 2016), Maize (Omer et al., 2017) and Sorghum (Chen et al., 2021). The impaired germination can be attributed to a number of reasons like reduced water uptake under high salinity (Migahid et al., 2019), damaged enzyme structure required for hydrolysis of seed reserve (Ibrahim 2016), increase in respiration rate during salinity stress due to shortage of metabolites required for embryo growth (Chen and Arora, 2011). A good germination percentage under higher salinity stress implies tolerance attributes in the variety GA-1.

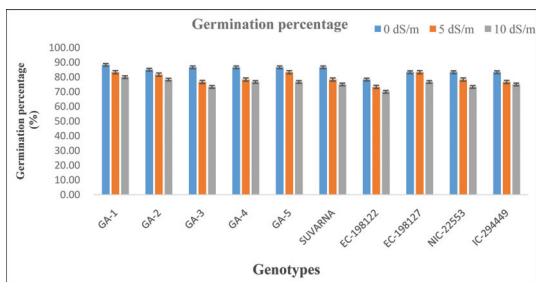


Figure 1. Germination percentage of *Amaranthus* genotypes under varying level of salinity stress

Speed of germination under salinity stress

Speed of germination decreases with increase in salinity level (Singh et al. 2012). Speed of germination is a trait which indicates fast germination of seed under stress and fast germination may be an indicator of tolerance. Significant variation with respect to genotypes and treatments in the present investigation was found (Fig. 2). Per cent reduction in speed of germination was 6.71 and 11.31 at 5 and 10 dS/m respectively which indicates that the salinity reduces the speed of germination.

The genotypes GA-1, GA-5, and EC-198127 reported the highest speed of germination at 5 dS/m, while EC-198122 recorded the slowest rates. The order of genotypes' speed of germination at 10 dS/m was GA-1, GA-2, GA-4, GA-5, SUVARNA, IC-

294449, GA-3, NIC-22553, and EC-198122. The decrease in speed of germination at higher salinity level might be due to increased osmotic pressure which interrupts the water uptake capacity of seed, since water is essential for hydrolysis and mobilisation of storage product as well as transport to growing embryo. Ionic stress may also be reason for reduction in speed of germination (Azeem et al., 2017), increasing salt concentration reduce the speed of germination (Belmehdi et al., 2018).

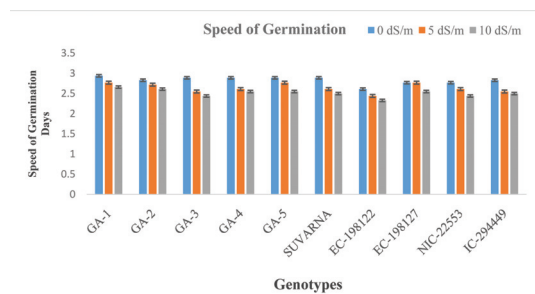


Figure 2. Speed of germination of *Amaranthus* genotypes under varying level of salinity stress

Growth assessment under salinity stress

Root and shoot length-Healthy growth of seedling is important for early plant establishment and further vegetative and reproductive developmental phases of the plant. *Brassica napus* seedlings' root and shoot length serves as a preliminary predictor of their susceptibility to salt (Long et al., 2013). In the current study, both root and shoot length declined as salinity levels rose; however, at 5 dS/m, shoot length decreased by a higher percentage than root length (9.69), whereas at 10 dS/m, root length decreased more than shoot length (Fig. 3 and 4). This indicates that grain amaranthus show tolerance to moderate salinity level through increased the root growth but higher salinity level (10 dS/m) further stop the growth of root. According to Munns (2002), root growth is less susceptible to salinity stress than shoot however, these responses are cultivar dependent (Zahra Rahnesan et al., 2018). In the current experiment under moderate stress, genotype GA-1, which is on par with genotype GA-3, recorded the largest root length, whereas genotype EC-198122 recorded the shortest value.

Additionally, GA-1 kept its longer root and shoot length at 10 dS/m (Figs. 3 and 4). With regard to genotypes, salinity level, and genotypes salt stress interaction, the mean shoot length of varied significantly (Fig. 3). The genotype GA-1, which is on par with SUVARNA, GA-5, recorded the highest value, whereas the genotype EC-198122, at a salinity level of 5 dS/m, recorded the lowest result. While, at 10 dS/m the maximum shoot length was recorded in genotype GA-1. On the basis of mean value shoot length followed the following pattern GA-1 > GA-2 = SUVARNA > GA-4 > GA-3 = GA-5 > EC-198127 > NIC-22553 > IC-294449 > EC-198122 at 10 dS/m. The root and shoot length of all genotypes reduced on average as salt level increased. Root and shoot lengths varied significantly based on genotype and salt level. Salt concentrations in an amaranthus crop greatly decreased the length of the shoot and the roots (Omamni, 2006) and sugarbeet (Ghoulam et al., 2002).

Seedling dry weight

The seedling dry weight varied significantly depending on the genotypes and treatments while

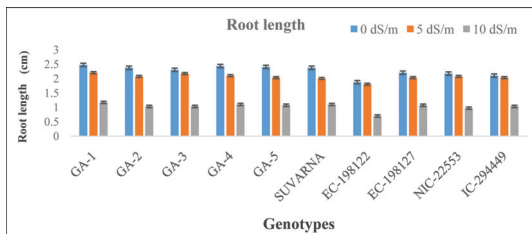


Figure 3. Root length of *Amaranthus* genotypes under varying level of salinity stress

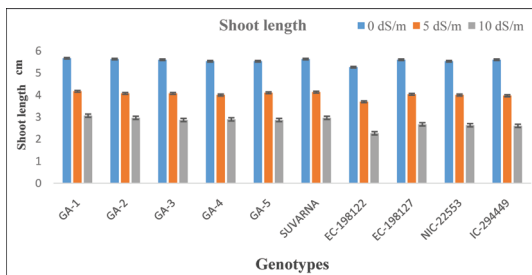


Figure 4. Shoot length of *Amaranthus* genotypes under varying level of salinity stress

interaction of $G \times T$ didn't differ significantly (Fig. 5). The per cent decrease in seedling dry weight is 25.33 and 40.17 at 5 dS/m and 10 dS/m respectively. Sorghum's dry weight decreased as a result of salinity stress (Chen et al., 2021). The initial dry matter of seedling depends on the seed storage. Hydrolysis of stored compound and its translocation to the growing parts is important function for growth of seedling. Water is essential for these two phenomenon which is reduced under salinity (Munns, 1993, Puvanitha and Mahendran 2017) therefore, the dry weight might decrease under salinity. Maintenance of growth under salinity is important for the tolerance. In the present study it was recorded that genotype GA-1 maintain maximum growth at 5 and 10 dS/m level. The pattern followed by genotypes in T_3 treatment was GA-1 = SUVARNA > GA-3 = GA-4 > GA-2 = GA-5 > NIC-22553 > EC-198127 > IC-294449 > EC-198122.

Identification of physiological traits confer salinity tolerance

Water content of seedlings and its relationship with root- shoot ratio

Maintenance of plant water status is important trait to confer salinity tolerance (Harris et al., 2010) The plants with high water content can efficiently maintain water status under salinity (Negrao et al., 2017). In the present investigation, the genotype GA-1 maintained higher water content both at 5 and 10 dSm⁻¹ (Fig. 6). This may be the reason for higher salinity tolerance index for GA-1 than other genotypes. Higher water content of GA-1 is also related with the higher root length under salinity

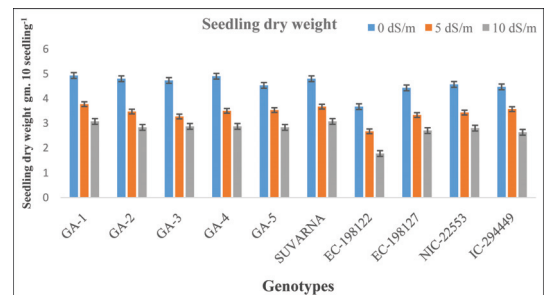


Figure 5. Seedling dry weight of *Amaranthus* genotypes under varying level of salinity stress

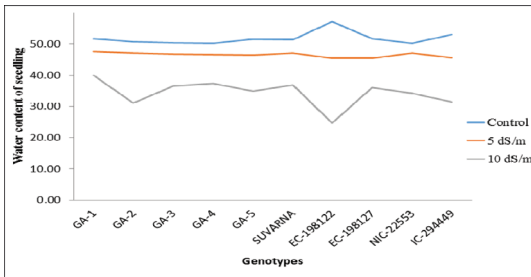


Figure 6: Water content (%) of grain amaranth genotypes under salinity stress

stress.

The water content of seedling showed negative correlation (0.5283) with the root shoot ratio under control conditions (fig 7a), while root-shoot ratio and water content had a positive connection at both salinity levels 5 dS/m (0.1691) and 10 dS.m (0.4850), as shown in fig 7b and 7c respectively. This indicates that under salinity stress, the increased root length might be an important trait for maintenance of water status of seedlings. Root length and water content of seedlings are thus important traits for screening of salinity tolerance genotypes.

Salt tolerance index of Amaranthus genotypes at moderate and high salinity level

Salt tolerance index (STI) values at 5 dS/m electrical conductivity indicated that GA-1, GA-5, SUVARNA and IC-294449 have greater capacity to produce dry matter than other genotypes (Table 2). The salt tolerance index of genotypes follows the pattern IC-294449 > GA-1 = GA-5 = SUVARNA > EC-198127 = NIC-22553 > GA-4 = EC-198122 > GA-2 > GA-3. At 10 dS/m, the dry matter

Figure 7. Correlation of water content in seedling with root shoot ratio of grain amaranth genotypes under control (a), 5 dS/m (b) and 10 dS/m (c) of salinity stress

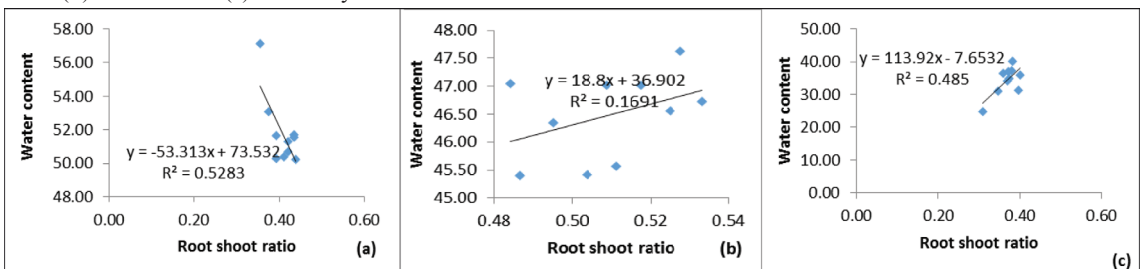


Table 2 . Salt tolerance index of *Amaranthus* genotypes under medium and high salinity stress at seedling stage based on seedling dry weight

| Genotypes | Salt tolerance index | |
|--------------------|---------------------------------|--------------------------------|
| | Medium salinity stress (5 dS/m) | High salinity stress (10 dS/m) |
| GA-1 | 0.77 | 0.62 |
| GA-2 | 0.72 | 0.60 |
| GA-3 | 0.69 | 0.61 |
| GA-4 | 0.73 | 0.59 |
| GA-5 | 0.77 | 0.63 |
| SUVARNA | 0.77 | 0.63 |
| EC-198122 | 0.73 | 0.48 |
| EC-198127 | 0.75 | 0.60 |
| NIC-22553 | 0.75 | 0.60 |
| IC-294449 | 0.79 | 0.58 |
| Mean | 0.75 | 0.59 |
| Standard deviation | 0.03 | 0.04 |

production capacity of IC- 294449 declined while GA-1 maintained maximum tolerance in terms of dry matter production and for this higher level of salinity, the pattern followed by genotypes was : GA-5 = SUVARNA > GA-1 > GA-3 > GA-2 = EC-198127 = NIC-22553 > GA-4 > IC-294449 > EC-198122. Salt tolerance index (STI) shown at 5 dS/m electrical conductivity indicates that GA-1, GA-5, Suvarna and IC-294449 have highest capacity to produce dry matter than other genotypes. However capacity of IC- 294449 for dry matter production at 10 dS/m decline but GA-1 maintained the maximum tolerance at highest salinity level (10 dS/ m).

Correlation of membership function value with root shoot ratio and seedling vigour index

Salt tolerance evaluation based on the comprehensive analysis of multiple traits and its tolerance indices is critical to identifying salt-

tolerant genotypes in *Brassica napus* (Hu et al., 2018). The genotype with higher MFV can be considered as tolerant to salinity (Ding et al., 2018, Wu et al., 2019). In light of above facts we can identify the traits that is correlated with higher MFV and ultimately better tolerance to salinity. In the current investigation, it was noted that higher root shoot ratio helps in maintenance of higher water status of seedling. The root shoot ratio was also recorded to be positively correlated with MFV under moderate (Fig. 8a) and high salinity stress (Fig. 8b). At 5 dS/m the correlation was non significant (0.0056) while at 10 dS/m the significant correlation (0.6366) was recorded. This suggests that one of crucial selection indices to assess genotypes salt tolerance is the root- shoot ratio. Seedling vigour is another important trait indicating the health of seedling under salt stress, influences plant population under salinity stress (Wahid et al. 2011, Chen et al., 2021). Germination and seedling vigour are the key attributes of plants during initial growth stage and are vital determinants of yield (Krishnamurthy et al., 2007, Bybordi and Tabatabaei, 2009, Rajabi et al., 2020). In the present investigation seedling vigour index decline with increase in salinity. However, it showed positive correlation with MFV at both salinity level (Fig. 8 c & d). This indicate that the vigour decline at higher salinity stress but it is positively correlated with the salinity tolerance.

Conclusions

In the current study, root-shoot ratio, seedling vigor, and seedling water content were the traits that conferred tolerance to salinity stress at the early seedling stage in genotypes of grain amaranth. The genotypes exhibiting greater values for these three indices also displayed greater germination percentage, seedling weight, vigour, STI and dry matter production during salinity stress. Root-shoot ratio and seedling vigour were further positively correlated to MFV implying that these indices altogether impart salinity tolerance to the genotypes with variable responses. The genotype GA-1 was

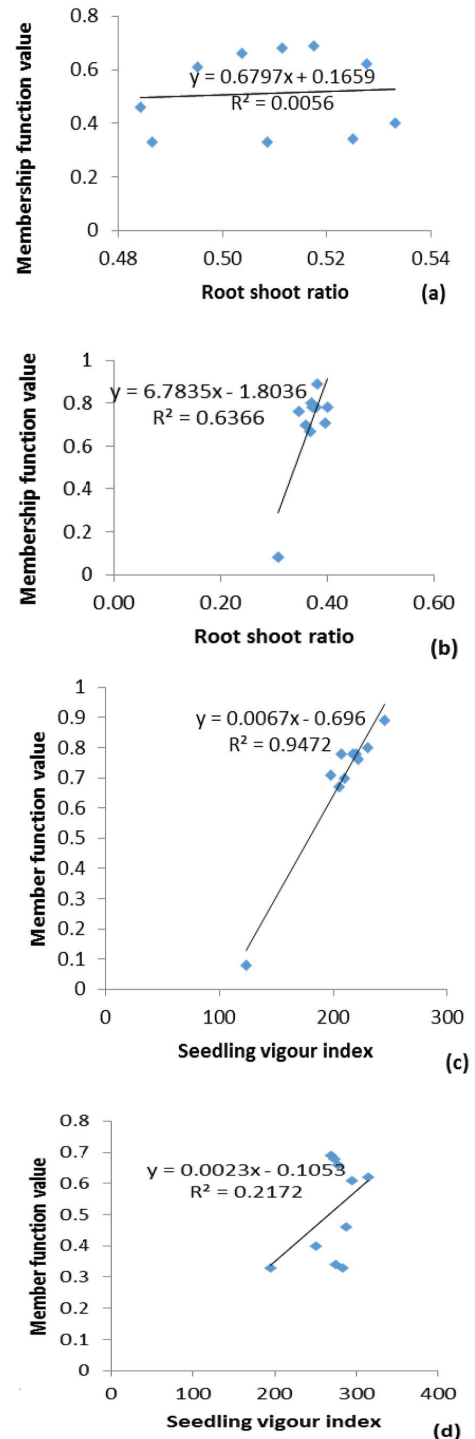


Figure 8. Correlation of membership function value with root shoot ratio (a and b) and seedling vigour index (c and d) of different grain amaranth genotypes at 5 dS^m⁻¹ (a and c) and 10 dS^m⁻¹ (b and d) salinity stress

found to be the best performing out of all the genotypes undertaken and hence might have better performance under salinity stress conditions.

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