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Assessment of Salinity Indices to Identify Mint Ecotypes using Intelligent and Regression Models

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Abstract

Despite recent development in producing chemical medicines, associated side effects have led to increased use of medicinal plants and natural compounds. Soil salinity, especially in arid and semi-arid regions, is a serious threat to global agriculture. Nowadays, efforts have been made to find benchmarks that can effectively select salt-tolerant or salt-resistant genotypes. In this regard, the use of computer software to predict the indices can help us for screening the most tolerant ecotypes. The objectives of the present study were to determine the best indicators of salinity tolerance using intelligent and regression models for eighteen commercial ecotypes of mint. The seedlings were planted in plastic pots and arranged in a split factorial experiment in a randomized complete block design with four replicates. The treatments consisted of four levels of salinity (0, 2.5, 5 and 7.5 dS m^{-1}), two levels of harvesting time, and 18 ecotypes. The plants were grown until the flowering stage and then harvested. There was a significant difference between ecotypes in terms of calculated indices at all three levels of salinity. Indicators such as TOL, MP, GMP, YSI, STI and HM showed a significant positive correlation with YS and YP at all three levels of salinity. The cluster analysis divided the ecotypes into three distinct groups based on the calculated indices at all levels of salinity. The principal component analysis revealed that the YP, YS, TOL, MP, GMP, YSI, STI and HM were more suitable among others salt stress indices. The sensitivity analysis at 2.5 dS m^{-1} salinity level showed that the HM, STI, YSI, YI, SSI and MP indices were of higher importance than the others. At 5 dS m^{-1} salinity level, the HM, STI, YSI, YI, GMP and MP indices showed the highest importance whereas at 7.5 dS m^{-1} salinity level, the STI, YSI, YI, GMP and YP indices indicated the highest importance. In general, the results suggest that $ANN_{(MLP)}$ model (R^2 = 0.999) is the best model to predict at all salinity levels. E13, E14, E15, E16 and E18 ecotypes are the most salt tolerant ecotypes which can be used for the future breeding program.

Keywords: Mint, predict, regression model, salinity

Abbreviations: YP: Yield in stress condition; YS: Yield in non-stress condition; TOL: Stress tolerance; MP: Mean productivity; GMP: Geometric mean productivity; SSI: Stress susceptibility index; YI: Yield index; YSI: Yield stability index; STI: Stress tolerance index; HM: Harmonic mean; ANFIS: Adaptive neuro fuzzy inference system; ANN: Artificial neural network; MLP: Multilayer perceptron; RBF: Radial basis function; GA: Genetic algorithm; OLS: Ordinary least squares; PCR: Principal component regression; PLS: Partial least squares; R^2: Coefficient of determination; VAF: Value account for; MAPE: Mean absolute percentage error; RMSE: Root mean square error; RPD: Relative percent difference.

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Introduction

According to the World Health Organization, medicinal plants are used by a large number of people, especially in developing countries (Bridge, 2016). Currently, 80% of the people in these countries use medicinal plant products to meet their medicine needs (Sukanya et al., 2009).

Mint (*Mentha spicata* L.) originally has been used as a medicinal herb to relieve stomach ache and chest pains. The economic importance of mints is also evident; mint oil and its constituents and derivatives are used as flavoring agents throughout the world in food, pharmaceutical, herbal, perfumery, and flavoring industries (Brahmi et al., 2017). Plants from this genus can be found in multiple and diverse environments, but most *Mentha* plants grows best in wet environments, moist soils and partial shade (Salehi et al., 2018). Different genera of this plant are widely cultivated in diverse areas of Iran such as Mazandaran, Gilan, Gorgan, Hazmazgan and southern Fars, Semnan, Kurdistan and Arak (Mozaffarian, 2008).

The genus *mentha* is one of the important members of the family Lamiaceae, a vast group of aromatic herbs of notable economic values due to its valuable essential oil. Recent data, based on morphological, cytological and genetic characteristics, have shown that genus *Mentha* can be classified into 42 species, 15 hybrids and hundreds of subspecies, varieties and cultivars (Tucker 2007; Salehi et al., 2018). Most *Mentha* species are perennial and fast-growing, extending their growth through a network of runners, contain essential oils, and are widely cultivated as industrial crops for essential oil production (Kumar et al., 2011).

Mint is also widely used in food and flavours as well as pharmaceutical and cosmetic industries. Approximately 10,000 tonnes of natural menthol and 2,000 tonnes of synthetic menthol are annually used by the pharmaceutical, cosmetic and cigarette industries across the globe (Yaseen et al., 2000; Annicchiarico, 2002; Lal, 2007, 2012). The plant is a rich source of essential oils that have a major nutritional and medicinal value, for example it has been used as an anti-inflammatory, anti-bacterial and digestive aid in traditional and modern medicine (Khalvandi et al., 2019; Mohkami et al., 2014).

Soil and water salinity are among the most important environmental factors limiting plants growth and production worldwide, especially in arid and semi-arid regions (Kachout et al., 2009). Currently, millions of hectares of agricultural land in the world are facing problems due to salinity (Dagar and Minhas, 2016). Salty lands are generated by the accumulation of soluble solutes such as chlorine, sulfate, bicarbonate, and sometimes nitrate, in particular sodium, calcium, magnesium and rarely potassium in non-saline soils (Shannon, 1997). In addition to cations, anions seem to also contribute to salinity, while sodium chloride and sodium sulfate play significant role in causing damage to plants due to their high solubility (Keutgen and Pawelzik, 2009).

To date, several efforts have been made to find benchmarks that can effectively select salt-tolerant or salt-resistant genotypes (Ashraf and Wu, 1994). However, the probability that the stress-tolerance genes in a plant are centralized and recognized by physiological methods is very limited (Flowers, 1997). Therefore, yield and yield components sustainability and stability under stress conditions are still among the main indicators of selection for finding tolerant genotypes in many breeding programs (Flowers and Yeo, 1995). Evaluation of plant's yield is the most important indicator for identifying compatible genotypes in stressed environments (Blum, 2005). There are different indices for assessing the stability of genotypes under various stress conditions (Dhanda et al., 2004). Stress tolerance index (STI) is a suitable criterion for selecting genotypes to achieve high performance under stress conditions. This index separates the genotypes of high performance in stressed and non-stressed conditions from other groups (Fernandez, 1992). The tolerance index (TOL) is obtained from the difference in performance under stressed and non-stress conditions. The higher values indicate lower genotype stability in different environments. In the stress susceptibility index (SSI), its lower values show a greater stability of a genotype under stress and non-stress conditions (Fischer and Maurer, 1978). Due to the high correlation between tolerance to stress condition and average yield in different environments, the Mean Productivity Index (MP) can be used as a suitable criterion for the selection of genotypes (Rosielle and Hamblin, 1981).

Following the rapid advancement of diverse sciences in the 20th century, non-analytic nonlinear functions were created in various engineering processes, requiring their numerical solution to evolve different numerical solution structures (Van Gorder, 2017). Genetic algorithms as one of these structures for the first time around three decades ago were inspired by natural structures (Bi et al., 2015). Hosseini et al. (2016) have stated that a genetic algorithm model could predict soil mechanical resistance more precisely than multiple regression with $R^2 = 0.90$ and RMSE = 0.34.

Since, ANN, ANFIS, GA, PLS, OLS and PCR models have not been used to evaluate stress tolerance indices to date, also the interest in studying mint for human beings is majorly related to its phytosanitary effects. The aim of this study was the application of these models as novel approach to determine the best indicators for salinity tolerance using intelligent and regression models for commercial mint ecotypes in order to be able to use them in the future breeding programs.

Materials and methods

The ecotypes were provided from the Gene Bank of Research Institute of Forests and Rangelands. The seeds were sown in plastic pots (25 cm diameter and 30 cm height) filled with cocopeat (40%) and perlite (60%) and placed in a glasshouse with a temperature between 25 and 28 °C, 16/8 day/night photoperiod and relative humidity of 60% throughout the experiment. The treatments consisted of four levels of salinity (0, 2.5, 5 and 7.5 dS m^{-1}), two levels of harvesting time and 18 ecotypes (Table 1), which arranged as split factorial experiment using randomized complete block design with four replications. The plants were watered with Hoagland nutrient solution (Hoagland and Arnon, 1950). Salinity was imposed 15 days after seed sowing. To prevent salinity shock to the seedlings 5 and 7.5 dS m^{-1} salinity levels were gradually applied (Reich et al., 2017), in a way that the electrical conductivity of the solution increased stepwise by 2.5 dS m^{-1} to reach 5 and 7.5 dS m^{-1}. Moreover, to prevent the accumulation of solutes in the culture medium, washing was done in certain periods. Eventually, harvesting was performed when the plants entered into the flowering phase. The plants were weighted and then placed in an oven at 70 °C for 48 h to determine dry weight using a digital scale.

The eight stress tolerance indices were calculated using the following equations:

$$TOL = Yp - Ys$$ (Hossain et al., 1990) (1)

$$MP = \frac{Ys + Yp}{2}$$ (Bouslama and Schapaugh, 1984) (2)

$$GMP = \sqrt{Ys \times Yp}$$ (Sio-Se Mardeh et al., 2006) (3)

$$SSI = \frac{1 - \frac{Ys}{Yp}}{1 - \frac{\overline{Ys}}{\overline{Yp}}}$$ (Fischer and Maure, 1978) (4)

$$YI = \frac{Ys}{Yp}$$ (Gavuzzi et al., 1997) (5)

$$YSI = \frac{Ys}{\overline{Yp}} \quad \text{(Bouslama and Schapaugh, 1984)} \quad (6)$$

$$STI = \frac{Ys \times Yp}{\overline{Yp}^2} \quad \text{(Gavuzzi et al., 1997)} \quad (7)$$

$$\frac{2(Ys \times Yp)}{(Ys + Yp)} \quad \text{(Rosielle and Hamblin, 1981)} \quad (8)$$

All equations are based on dry matter yield in stressed (Ys) and non-stressed (Yp) conditions and average dry matter yield in stressed ($\overline{Ys}$) and non-stressed ($\overline{Yp}$) conditions.

Intelligent methods

Artificial neural network (ANN)

In this study, the network was designed with 10 nodes in the input layer and 1 node in the output layer. The best transmission function was Tansig for the structure of the neural network (Fig. 1) (Hosseini et al., 2017). For neural network training, MATLAB 7.6 utilized the Multi-Layer Perceptron (MLP) and Radial Basis Function (RBF) network.

Training processes involved weight rectification between diverse layers until minimum difference was achieved between real and predicted data. Lastly, the best network structure was carefully chosen based on minimum root mean square error (RMSE) and maximum R^2 (Liu et al., 2001).

Adoptive neuro-fuzzy inference system (ANFIS)

An additional soft computing technique used in natural science is neuro-fuzzy modeling (Iphar et al., 2008; Singh et al., 2007). A neuro-fuzzy system is, in fact, a neural network that is functionally equivalent to the fuzzy inference model. Jang (1993) suggested a fuzzy logic model named ANFIS which employs some properties of an ANN such as learning and parallelism. The basic structure of an ANFIS model was given by Padmini et al. (2008) (Fig. 2).

Genetic algorithm (GA)

Genetic algorithms (GA) are mathematical models of natural genetics where the power of nature to develop, destroy, improve and annihilate life is abstracted and used to explain complex optimization complications (Fig. 3).

Holland (1975) established this prevailing technique and it has been applied in several fields of science.

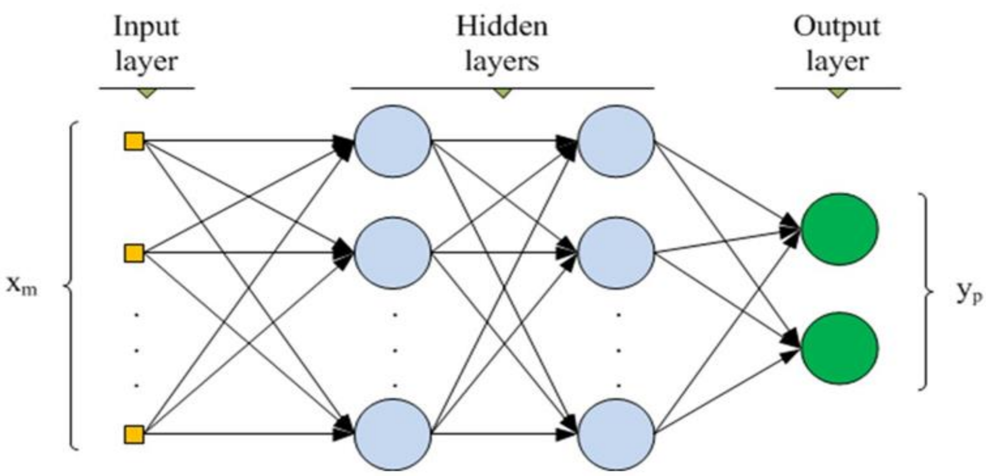

Fig. 1. A simple multilayer of Artificial Neural Network (ANN) configuration

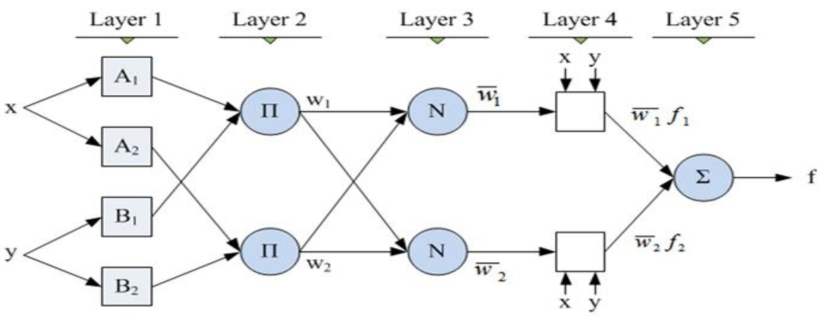

Fig. 2. Basic adaptive neuro-fuzzy inference system (ANFIS) structure.

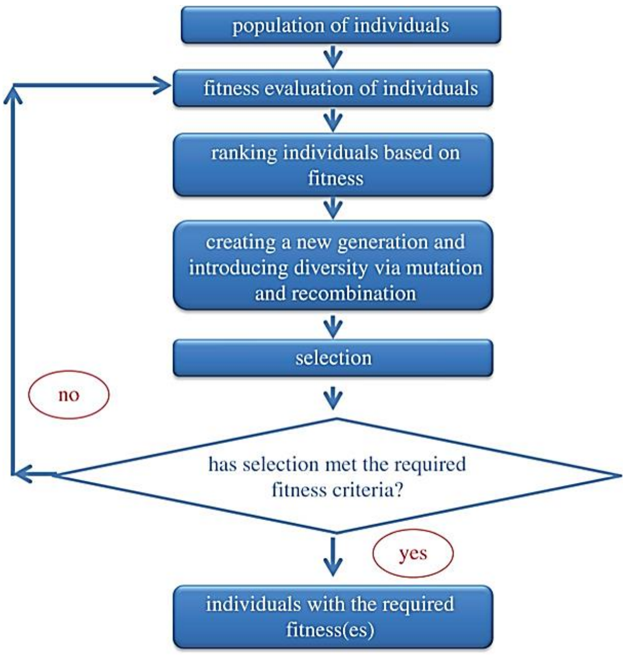

Fig. 3. Flowchart of a single population evolutionary genetic algorithm (GA)

Regression methods

Partial least squares regression (PLS), ordinary least-squares regression (OLS) and principal components regression (PCR). PLS, OLS, and PCR are three techniques to model a response or dependent variable when there are several predictors or independent variables existing, and the predictor variables are extremely correlated (Hosseini et al., 2017).

Model evaluation

To evaluate the proficiency of models, several statistical standards such as value account for (VAF), root mean square error (RMSE), R^2, mean absolute percentage error (MAPE) and relative percent difference (RPD) (only for PLS, PCR and OLS) were used as follows:

$$VAF = \left[1 - \frac{Var(P(si) - M(si))}{Var(M(si)}\right] \times 100 \tag{9}$$

$$RMSE = \sqrt[2]{\left\{\frac{\sum[P(si) - M(si)]^2}{n}\right\}} \tag{10}$$

$$R^2 = 1 - \frac{\sum_{i=1}^{n}[M(si) - P(si)]^2}{\sum_{i=1}^{n}[M(si) - A(si)]^2} \tag{11}$$

P (si), M (si) and A (si) represent the amounts of predicted, measured and average dry matter yields and represents the number of sampling points. VAF (Eq. (9)) and RMSE (Eq. (10)) indices were computed to evaluate the proficiency of the predictive model developed in the study as employed by Grima and Babuska (1999), Gokceoglu (2002), and Yilmaz and Yuksek (2008). If the VAF is 100 and RMSE is 0, then model proficiency was outstanding. R^2 (Eq. (11)) is also commonly used in intelligent methods to evaluate model proficiency. Mean absolute percentage error (MAPE), which is a measure of accuracy in a fitted series value in statistics, was also used for comparison of the prediction proficiency of the models. MAPE usually represent accuracy as a percentage (Eq. (12)).

$$MAPE = \frac{\sum_i^N \left|\frac{Ai - Pi}{Ai}\right|}{N} \times 100 \qquad (12)$$

Where Ai represents the actual value and Pi represents the predicted value. The achieved values of ME, VAF, RMSE and MAPE were used to demonstrate prediction proficiency. Viscarra Rossel et al. (2009) proposed that relative percent difference (RPD; Eq. (13)) can be used to evaluate model proficiency; RPD values which employed to evaluate the models are shown in Table 2.

$$RPD = \frac{SD}{RMSEp} \qquad (13)$$

Where SD is standard deviation.

Statistical analysis

The data were separated into a training data subset (70%) and testing data subset (30%). Data subsets were used for defining the proficiency of seven methods; GA, ANN, ANFIS, PLS, PCR and OLS. Matlab (MathWorks, Natick, MA) was used to analyze GA, ANN and ANFIS and XLSTAT (Add-In-Soft, Paris, France) were used for PLS, OLS, PCR, cluster analysis and principal component analysis.

Results

Analysis of variance and correlation

The analysis of variance indicated that there was a significant difference between ecotypes based on all studied indices (Table 1, 2, and 3). Relative percent difference (RPD) values for evaluating models is presented in Table 4.

The results also showed that there was a significant difference between ecotypes in terms of calculated indices at all salinity levels. The TOL, MP, GMP, YSI, STI and HM showed a significant and positive correlation with both YS and YP at all salinity levels (Tables 5, 6 and 7).

Table 1. Ecotype number, species, latitude, longitude, altitude and place related to 18 mint ecotypes

Ecotype number	Species	Place	Latitude (N)	Longitude (E)	Altitude (m)
E_1	*longifolia*	Mazandaran-Hezar Jarib	36◦4524"	53◦2806"	389
E_2	*longifolia*	Fars-Shiraz	29◦7447"	52◦4590"	1491
E_3	*longifolia*	Kordestan-Sanandaj, Dolatabad Village	35◦2000"	47◦9999"	2039
E_4	*longifolia*	Markazi-Arak	34◦1115"	49◦3111"	2030
E_5	*longifolia*	Golestan-Ramyan	36◦5513"	55◦0655"	780
E_6	*longifolia*	Tehran	35◦5229"	52◦9383"	1940
E_7	*longifolia*	Zanjan	36◦4235"	48◦0703"	1800
E_8	*longifolia*	Semnan-Damghan	36◦2748"	54◦1738"	2300
E_9	*longifolia*	Ilam-Dehloran	32◦5307"	47◦0030"	807
E_{10}	*pulegium*	Markazi-Tafresh	34◦4703"	49◦5800"	1550
E_{11}	*pulegium*	Markazi-Khomein	33◦3635"	50◦0150"	1808
E_{12}	*pulegium*	South Khorasan-Sarbisheh	32◦3223"	65◦1139"	1817
E_{13}	*pulegium*	Mazandaran	32◦3703"	51◦2590"	1662
E_{14}	*spicata*	Isfahan-Najafabad	32◦1854"	51◦4307"	1652
E_{15}	*spicata*	Yazd	31◦1588"	54◦3089"	1243
E_{16}	*rotundifolia*	Ilam-Ivan	33◦5108"	46◦1104"	1142
E_{17}	*mozafariani*	Hormozgan-Bandar Abbas	27◦5014"	56◦1805"	1117
E_{18}	*piperita*	Mazandaran-Sari	36◦7700"	53◦0599"	1255

Table 2. The results of analysis related to yield in stress condition (YP), yield in non-stress condition (YS), stress tolerance (TOL), mean productivity (MP), geometric mean productivity (GMP and stress susceptibility index (SSI) indices at different salinity stress levels

Stress level (dS. m^{-1})	S.O.V	YP	YS	TOL	MP	GMP	SSI
2.5	Block	0.40^{ns}	0.08^{ns}	0.20^{ns}	0.50^{ns}	0.06^{ns}	0.01^{ns}
	Treatment	20.63**	14.05**	1.48**	16.97**	16.81**	0.14**
	Error	0.13	0.05	0.15	0.05	0.05	0.02
	CV (%)	4.37	3.61	20.36	3.16	3.08	17.34
5	Block	0.07^{ns}	0.02^{ns}	0.15^{ns}	0.08^{ns}	0.01^{ns}	0.008^{ns}
	Treatment	21.15**	10.16**	2.67**	14.98**	14.46**	0.043**
	Error	0.09	0.01	0.10	0.02	0.02	0.004
	CV (%)	3.69	1.94	10.78	2.44	2.18	6.82
7.5	Block	0.07^{ns}	0.02^{ns}	0.02^{ns}	0.04^{ns}	0.03^{ns}	0.0003^{ns}
	Treatment	21.15**	6.05**	5.30**	12.27**	11.09**	0.0026**
	Error	0.09	0.05	0.11	0.04	0.04	0.0023
	CV (%)	3.69	5.39	7.65	3.41	3.66	4.86

^{ns} and **: are non-significant and significant at 1% probability levels, respectively.

Table 3. The results of variance analysis related to yield index (YI), yield stability index (YSI), stress tolerance index (STI), harmonic mean (HM) and Ys-Yp indices at different salinity stress levels

Stress level (dS. m^{-1})	S.O.V	YI	YSI	STI	HM	Ys-Yp
2.5	Block	0.0004^{ns}	0.0011^{ns}	0.0036^{ns}	0.06^{ns}	0.02^{ns}
	Treatment	0.0081**	0.1941**	0.7997**	16.65**	1.48**
	Error	0.0016	0.0007	0.0028	0.04	0.15
	CV (%)	5.30	3.62	6.61	3.03	20.36
5	Block	0.001^{ns}	0.0002^{ns}	0.0001^{ns}	0.003^{ns}	0.15^{ns}
	Treatment	0.005**	0.1404**	0.5971**	13.975**	2.67**
	Error	0.001	0.0001	0.0010	0.017	0.10
	CV (%)	3.67	1.94	4.57	1.97	10.78
7.5	Block	0.0001^{ns}	0.0003^{ns}	0.001^{ns}	0.03^{ns}	0.02^{ns}
	Treatment	0.0032**	0.0836**	0.350**	10.06**	5.30**
	Error	0.0006	0.0006	0.001	0.05	0.11
	CV (%)	5.09	5.39	7.93	4.14	7.65

^{ns} and **: are non-significant and significant at 1% probability levels, respectively.

Table 4. Relative percent difference (RPD) values for evaluating models

RPD	Model situation
< 1.0	Very poor models/predictions
1.0 < RPD < 1.4	Poor models/predictions
1.4 < RPD < 1.8	Fair models/predictions
1.8 < RPD < 2.0	Good models/predictions
2.0 < RPD < 2.5	Very good quantitative models/predictions
RPD > 2.5	Excellent models/predictions

Table 5. Correlation matrix showing relationship between different salt tolerance indices of mint ecotypes at 2.5 dS. m^{-1} salt stress level

Indices	YP	YS	TOL	MP	GMP	SSI	YI	YSI	STI	HM
Yield in stress condition (YP)	**1**									
Yield in non-stress condition (YS)	0.97**	**1**								
Stress tolerance (TOL)	0.73**	0.56*	**1**							
Mean productivity (MP)	0.99**	0.99**	0.66**	**1**						
Geometric mean productivity (GMP)	0.99**	0.99**	0.64**	0.99**	**1**					
Stress susceptibility index (SSI)	-0.20	-0.41	0.49*	-0.30	-0.31	**1**				
Yield index (YI)	0.20	0.41	-0.48*	0.30	0.31	-0.99**	**1**			
Yield stability index (YSI)	0.97**	0.99**	0.56*	0.99**	0.99**	-0.41	0.41	**1**		
Stress tolerance index (STI)	0.98**	0.99**	0.62**	0.99**	0.99**	-0.32	0.32	0.99**	**1**	
Harmonic mean (HM)	0.99**	0.99**	0.63**	0.99**	0.99**	-0.32	0.32	0.99**	0.99**	**1**

* Significant at $p \leq 0.05$.** Significant at $p \leq 0.01$.

Table 6. Correlation matrix showing relationship between different salt tolerance indices of mint ecotypes at 5 dS. m^{-1} salt stress level

Indices	YP	YS	TOL	MP	GMP	SSI	YI	YSI	STI	HM
Yield in stress condition (YP)	**1**									
Yield in non-stress condition (YS)	0.98^{**}	**1**								
Stress tolerance (TOL)	0.91^{**}	0.80^{**}	**1**							
Mean productivity (MP)	0.99^{**}	0.99^{**}	0.87^{**}	**1**						
Geometric mean productivity (GMP)	0.99^{**}	0.99^{**}	0.86^{**}	0.99^{**}	**1**					
Stress susceptibility index (SSI)	-0.18	-0.38	0.24	-0.26	-0.28	**1**				
Yield index (YI)	0.18	0.38	-0.24	0.26	0.28	-0.99^{**}	**1**			
Yield stability index (YSI)	0.98^{**}	0.99^{**}	0.80^{**}	0.99^{**}	0.99^{**}	-0.38	0.38	**1**		
Stress tolerance index (STI)	0.98^{**}	0.99^{**}	0.83^{**}	0.99^{**}	0.99^{**}	-0.30	0.30	0.99^{**}	**1**	
Harmonic mean (HM)	0.99^{**}	0.99^{**}	0.84^{**}	0.99^{**}	0.99^{**}	-0.30	0.30	0.99^{**}	0.99^{**}	**1**

* Significant at $p \leq 0.05$.** Significant at $p \leq 0.01$.

Table 7. Correlation matrix showing relationship between different salt tolerance indices of mint ecotypes at 7.5 dS. m^{-1} salt stress level

Indices	YP	YS	TOL	MP	GMP	SSI	YI	YSI	STI	HM
Yield in stress condition (YP)	**1**									
Yield in non-stress condition (YS)	0.97^{**}	**1**								
Stress tolerance (TOL)	0.96^{**}	0.86^{**}	**1**							
Mean productivity (MP)	0.99^{**}	0.99^{**}	0.93^{**}	**1**						
Geometric mean productivity (GMP)	0.99^{**}	0.99^{**}	0.92^{**}	0.99^{**}	**1**					
Stress susceptibility index (SSI)	-0.24	-0.47^{*}	0.03	-0.32	-0.36	**1**				
Yield index (YI)	0.24	0.47^{*}	-0.03	0.32	0.36	-0.99^{**}	**1**			
Yield stability index (YSI)	0.97^{**}	0.99^{**}	0.86^{**}	0.99^{**}	0.99^{**}	-0.47^{*}	0.47^{*}	**1**		
Stress tolerance index (STI)	0.98^{**}	0.99^{**}	0.89^{**}	0.99^{**}	0.99^{**}	-0.40	0.40	0.99^{**}	**1**	
Harmonic mean (HM)	0.98^{**}	0.99^{**}	0.90^{**}	0.99^{**}	0.99^{**}	-0.40	0.40	0.99^{**}	0.99^{**}	**1**

* Significant at $p \leq 0.05$.** Significant at $p \leq 0.01$.

Therefore, selection was carried out based on the high values of these indices to select ecotypes with high performance in both normal and salt-stress conditions, such as ecotypes including E13, E14, E15, E16 and E18 (Tables 8, 9 and 10).

Table 8. Mean of yield in stress condition (YP), yield in non-stress condition (YS), stress tolerance (TOL), mean productivity (MP), geometric mean productivity (GMP, stress susceptibility index (SSI), yield index (YI), yield stability index (YSI), stress tolerance index (STI) and harmonic mean (HM) of 18 mint ecotypes at 2.5 dS. m^{-1} salt stress level

Group no. in dendrogram	Ecotypes	YP	YS	TOL	MP	GMP	SSI	YI	YSI	STI	HM	Ys-Yp
2	E_1	9.46	6.90	2.57	8.18	8.08	1.15	0.73	0.81	0.91	7.97	-2.57
3	E_2	6.05	4.32	1.73	5.18	5.11	1.21	0.72	0.51	0.36	5.04	-1.73
3	E_3	6.31	4.39	1.92	5.35	5.26	1.29	0.70	0.52	0.38	5.17	-1.92
3	E_4	5.45	4.07	1.38	4.76	4.71	1.07	0.75	0.48	0.31	4.66	-1.38
2	E_5	8.40	6.36	2.04	7.38	7.30	1.03	0.76	0.75	0.74	7.23	-2.04
3	E_6	5.70	4.44	1.26	5.07	5.03	0.94	0.78	0.52	0.35	4.99	-1.26
3	E_7	6.85	5.06	1.79	5.95	5.88	1.11	0.74	0.59	0.48	5.82	-1.79
2	E_8	9.21	6.62	2.59	7.92	7.81	1.19	0.72	0.78	0.84	7.70	-2.59
3	E_9	6.89	5.23	1.66	6.06	6.00	1.01	0.76	0.61	0.50	5.94	-1.66
2	E_{10}	7.78	6.35	1.42	7.06	7.03	0.77	0.82	0.75	0.68	6.99	-1.42
3	E_{11}	7.14	5.90	1.24	6.52	6.49	0.73	0.83	0.69	0.58	6.46	-1.24
3	E_{12}	7.35	5.94	1.41	6.64	6.60	0.81	0.81	0.70	0.60	6.56	-1.41
1	E_{13}	11.65	9.27	2.38	10.46	10.39	0.87	0.80	1.09	1.49	10.32	-2.38
1	E_{14}	10.55	8.28	2.27	9.42	9.35	0.91	0.79	0.97	1.21	9.28	-2.27
1	E_{15}	11.47	8.90	2.57	10.18	10.10	0.95	0.78	1.05	1.41	10.02	-2.57
1	E_{16}	12.17	8.49	3.68	10.33	10.16	1.28	0.70	1.00	1.43	10.00	-3.68
2	E_{17}	8.33	6.08	2.26	7.21	7.12	1.15	0.73	0.71	0.70	7.03	-2.26
1	E_{18}	12.38	10.52	1.86	11.45	11.41	0.64	0.85	1.24	1.80	11.37	-1.86
LSD 5%		0.44	0.23	0.42	0.28	0.27	0.17	0.04	0.03	0.06	0.25	0.42

Table 9. Mean of yield in stress condition (YP), yield in non-stress condition (YS), stress tolerance (TOL), mean productivity (MP), geometric mean productivity (GMP, stress susceptibility index (SSI), yield index (YI), yield stability index (YSI), stress tolerance index (STI) and harmonic mean (HM) of 18 mint ecotypes at 5 dS. m^{-1} salt stress level

Group no. in dendrogram	Ecotypes	YP	YS	TOL	MP	GMP	SSI	YI	YSI	STI	HM	Ys-Yp
2	E_1	9.46	6.12	3.35	7.79	7.60	1.00	0.65	0.72	0.80	7.41	-3.35
1	E_2	6.05	3.84	2.20	4.95	4.82	1.04	0.64	0.45	0.32	4.70	-2.20
1	E_3	6.31	4.07	2.23	5.19	5.07	1.01	0.65	0.48	0.36	4.95	-2.23
1	E_4	5.45	3.64	1.82	4.55	4.45	0.95	0.67	0.43	0.27	4.36	-1.82
1	E_5	8.40	5.25	3.15	6.82	6.64	1.07	0.63	0.62	0.61	6.46	-3.15
1	E_6	5.70	3.51	2.19	4.61	4.47	1.10	0.62	0.41	0.28	4.35	-2.19
1	E_7	6.85	4.16	2.68	5.50	5.34	1.12	0.61	0.49	0.39	5.18	-2.68
2	E_8	9.21	6.14	3.07	7.68	7.52	0.95	0.67	0.72	0.78	7.37	-3.07
1	E_9	6.89	4.25	2.63	5.57	5.41	1.09	0.62	0.50	0.41	5.26	-2.63
1	E_{10}	7.78	5.43	2.35	6.60	6.50	0.86	0.70	0.64	0.58	6.39	-2.35
1	E_{11}	7.14	4.92	2.22	6.03	5.92	0.89	0.69	0.58	0.48	5.82	-2.22
1	E_{12}	7.35	5.05	2.30	6.20	6.09	0.89	0.69	0.59	0.51	5.99	-2.30
3	E_{13}	11.65	7.87	3.78	9.76	9.57	0.93	0.68	0.92	1.27	9.39	-3.78
2	E_{14}	10.55	6.83	3.73	8.69	8.49	1.01	0.65	0.80	1.00	8.29	-3.73
3	E_{15}	11.47	7.19	4.28	9.33	9.08	1.07	0.63	0.84	1.14	8.84	-4.28
3	E_{16}	12.17	7.39	4.78	9.78	9.48	1.12	0.61	0.87	1.24	9.19	-4.78
1	E_{17}	8.33	4.98	3.35	6.66	6.44	1.15	0.60	0.59	0.57	6.24	-3.35
3	E_{18}	12.38	9.00	3.38	10.69	10.55	0.78	0.73	1.06	1.54	10.42	-3.38
LSD 5%		0.44	0.16	0.46	0.24	0.21	0.10	0.03	0.02	0.04	0.18	0.46

Table 10. Mean of yield in stress condition (YP), yield in non-stress condition (YS), stress tolerance (TOL), mean productivity (MP), geometric mean productivity (GMP, stress susceptibility index (SSI), yield index (YI), yield stability index (YSI), stress tolerance index (STI) and harmonic mean (HM) of 18 mint ecotypes at 7.5 dS. m^{-1} salt stress level

Group no. in dendrogram	Ecotypes	YP	YS	TOL	MP	GMP	SSI	YI	YSI	STI	HM	Ys-Yp
2	E_1	9.46	4.42	5.05	6.94	6.45	1.04	0.47	0.52	0.58	5.99	-5.05
3	E_2	6.05	2.88	3.17	4.46	4.17	1.03	0.48	0.34	0.24	3.90	-3.17
3	E_3	6.31	2.87	3.43	4.59	4.26	1.07	0.46	0.34	0.25	3.95	-3.43
3	E_4	5.45	2.65	2.80	4.05	3.80	1.01	0.49	0.31	0.20	3.57	-2.80
2	E_5	8.40	4.08	4.31	6.24	5.86	1.01	0.49	0.48	0.47	5.49	-4.31
3	E_6	5.70	2.72	2.98	4.21	3.94	1.02	0.48	0.32	0.21	3.68	-2.98
3	E_7	6.85	3.27	3.58	5.06	4.73	1.02	0.48	0.38	0.31	4.42	-3.58
2	E_8	9.21	4.49	4.72	6.85	6.43	1.00	0.49	0.53	0.57	6.04	-4.72
3	E_9	6.89	3.22	3.67	5.06	4.71	1.04	0.47	0.38	0.31	4.39	-3.67
3	E_{10}	7.78	3.87	3.90	5.82	5.49	0.98	0.50	0.46	0.42	5.17	-3.90
3	E_{11}	7.14	3.63	3.51	5.38	5.09	0.96	0.51	0.43	0.36	4.81	-3.51
3	E_{12}	7.35	3.74	3.61	5.55	5.24	0.96	0.51	0.44	0.38	4.95	-3.61
1	E_{13}	11.65	5.78	5.87	8.71	8.20	0.99	0.50	0.68	0.93	7.72	-5.87
1	E_{14}	10.55	4.92	5.64	7.73	7.20	1.05	0.47	0.58	0.72	6.70	-5.64
1	E_{15}	11.47	5.57	5.89	8.52	7.99	1.01	0.49	0.66	0.88	7.50	-5.89
1	E_{16}	12.17	5.44	6.73	8.80	8.13	1.08	0.45	0.64	0.91	7.51	-6.73
2	E_{17}	8.33	4.31	4.03	6.32	5.99	0.95	0.52	0.51	0.50	5.68	-4.03
1	E_{18}	12.38	7.13	5.25	9.76	9.40	0.83	0.58	0.84	1.22	9.05	-5.25
LSD 5%		0.44	0.31	0.46	0.31	0.31	0.07	0.03	0.04	0.06	0.32	0.46

Cluster and principal component analysis

Cluster analysis was attained by the similarity matrix based on Euclidean distance measurement and non-weighted paired group method using arithmetic average (UPGMA). The matrix of similarity was used for the cluster analysis (average of four replicates per ecotype). The cluster analysis divided the ecotypes into three distinct groups based on the calculated indices at all salinity levels (Fig. 4).

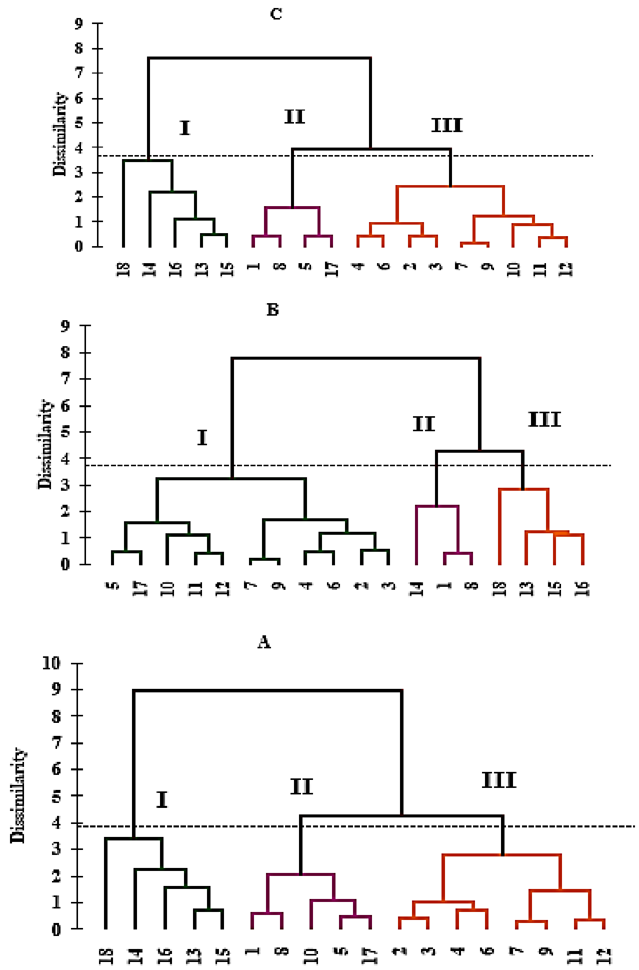

Fig. 4. Dendrogram based on UPGMA method for tolerance indices (YP: Yield in stress condition; YS: Yield in non-stress condition; TOL: Stress tolerance; MP: Mean productivity; GMP: Geometric mean productivity; SSI: Stress susceptibility index; YI: Yield index; YSI: Yield stability index; STI: Stress tolerance index; HM: Harmonic mean) in 18 mint ecotypes. A: (at 2.5 dS. m^{-1} salt stress level) B: (at 5 dS. m^{-1} salt stress level) and C: (at 7.5 dS. m^{-1} salt stress level)

The numbers 13, 14, 15, 16 and 18 ecotypes were placed in the first group at 2.5 and 7.5 dS m^{-1} levels. At 5 dS m^{-1} salinity level, numbers 13, 15, 16 and 18 ecotypes were placed in the third group. The above-mentioned ecotypes had a higher TOL, MP, GMP, YSI, STI and HM rate than the others.

Furthermore, the results of principal component analysis at different salinity levels showed that the first two principal components had the highest amount of relative variance of the total variation in yield performance and the measured indices. At all salinity levels, the YP, YS, TOL, MP, GMP, YSI, STI and HM indices showed the highest value in the first component. In addition, in the second component, the SSI and YI indices showed the highest rates. The relative variance for the first component at 2.5, 5 and 7.5 dS m^{-1} salinity levels were found to be 75.8%, 78.6% and 80.8%, respectively (Table 11).

Besides, in the second component, the relative variance was 23.7%, 21.2%, and 19%, respectively. The results indicated that the relative variance of the first component increased with increasing salinity level. By contrast, the amount of the second component decreased with increasing salinity levels. The Biplot chart shows the distribution of ecotypes around the evaluated indicators (Fig. 5).

Table 11. Principal component analysis based all salinity indices at three salt stress levels

Indices	Stress levels					
	2.5 (dS. m^{-1})		5 (dS. m^{-1})		7.5 (dS. m^{-1})	
	PC_1	PC_2	PC_1	PC_2	PC_1	PC_2
Yield in stress condition (YP)	0.987	0.157	0.989	0.147	0.978	0.205
Yield in non-stress condition (YS)	0.997	-0.063	0.997	-0.063	0.999	-0.045
Stress tolerance (TOL)	0.617	0.771	0.835	0.538	0.886	0.457
Mean productivity (MP)	0.998	0.058	0.998	0.061	0.993	0.119
Geometric mean productivity (GMP)	0.999	0.043	0.999	0.039	0.997	0.077
Stress susceptibility index (SSI)	-0.355	0.932	-0.319	0.947	-0.433	0.901
Yield index (YI)	0.356	-0.932	0.320	-0.947	0.433	-0.901
Yield stability index (YSI)	0.997	-0.062	0.997	-0.064	0.999	-0.045
Stress tolerance index (STI)	0.994	0.027	0.995	0.019	0.994	0.036
Harmonic mean (HM)	0.999	0.029	1.000	0.017	0.999	0.035
Relative variance (%)	75.799	23.728	78.527	21.179	80.793	19.005
Cumulative variance (%)	75.799	99.527	78.527	99.706	80.793	99.798

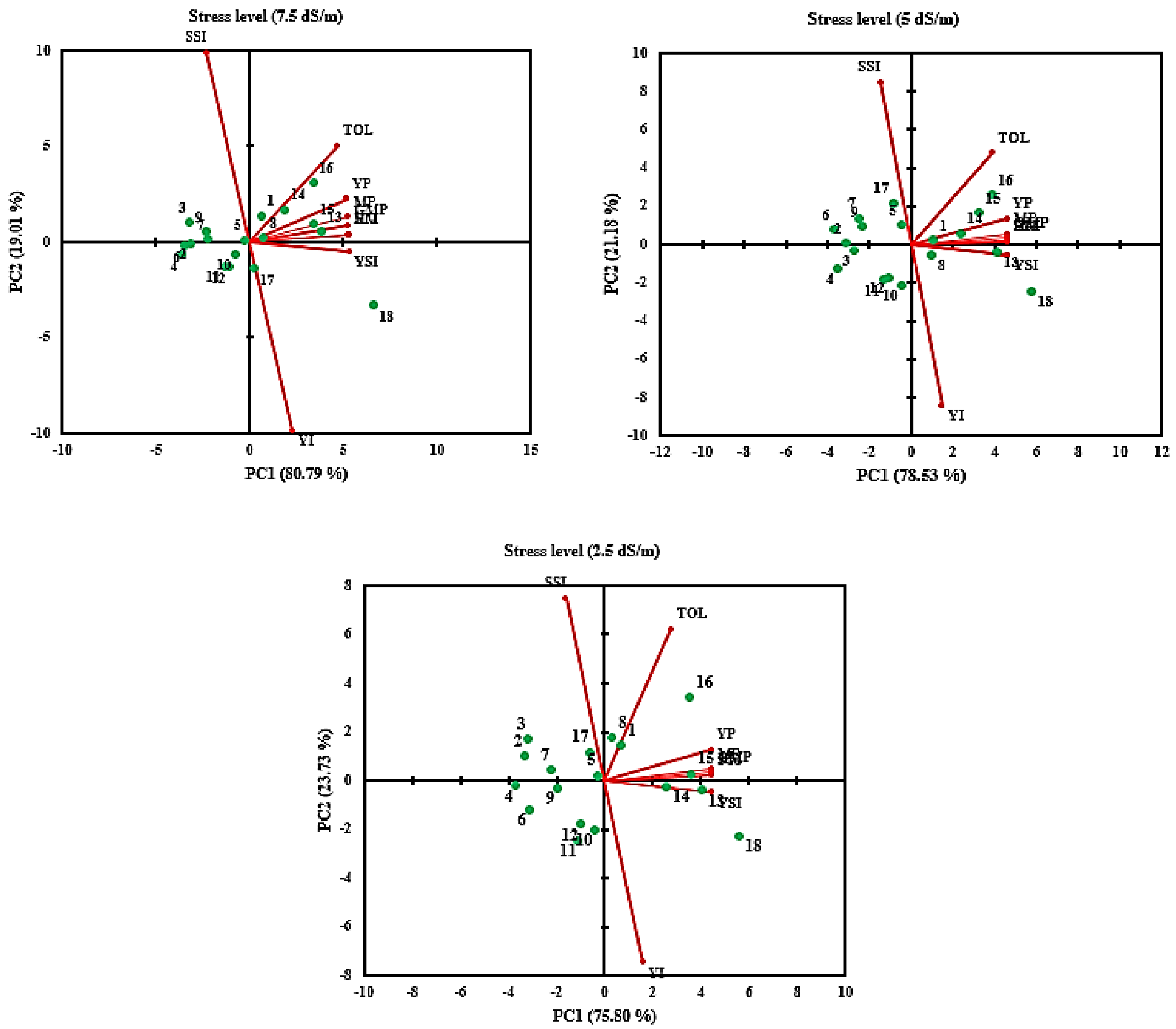

Fig. 5. The biplot chart based on Yp, Ys and eight salinity indices at three salt stress levels. [YP: Yield in stress condition; YS: Yield in non-stress condition; TOL: Stress tolerance; MP: Mean productivity; GMP: Geometric mean productivity; SSI: Stress susceptibility index; YI: Yield index; YSI: Yield stability index; STI: Stress tolerance index; HM: Harmonic mean]

Sensitivity analysis

The results of the sensitivity analysis at different levels of salinity using ANN is showed in Figure 6. The network with the highest RMSE independent input variable showed the most impact on the model.

To evaluate the sensitivity of the parameters, YP, TOL, MP, GMP, SSI, YI, YSI, STI and HM were considered as the inputs of the model. The results of the sensitivity analysis at 2.5 dS m^{-1} salinity showed that the HM, STI, YSI, YI, SSI and MP indices are of higher importance than the others. It was also observed that with increasing salinity level, the importance of indices would change greatly. Therefore, at the 5 dS m^{-1} salinity level, the HM, STI, YSI, YI, GMP and MP indices showed the most importance. The results of the sensitivity analysis at 7.5 dS m^{-1} salinity level differed from the lower salinity levels. Accordingly, the STI, YSI, YI, GMP and YP indicators were of the highest importance. Therefore, based on the results of the sensitivity analysis, the indices with higher importance were selected and evaluated as the input parameters in different models for prediction of dry matter performance.

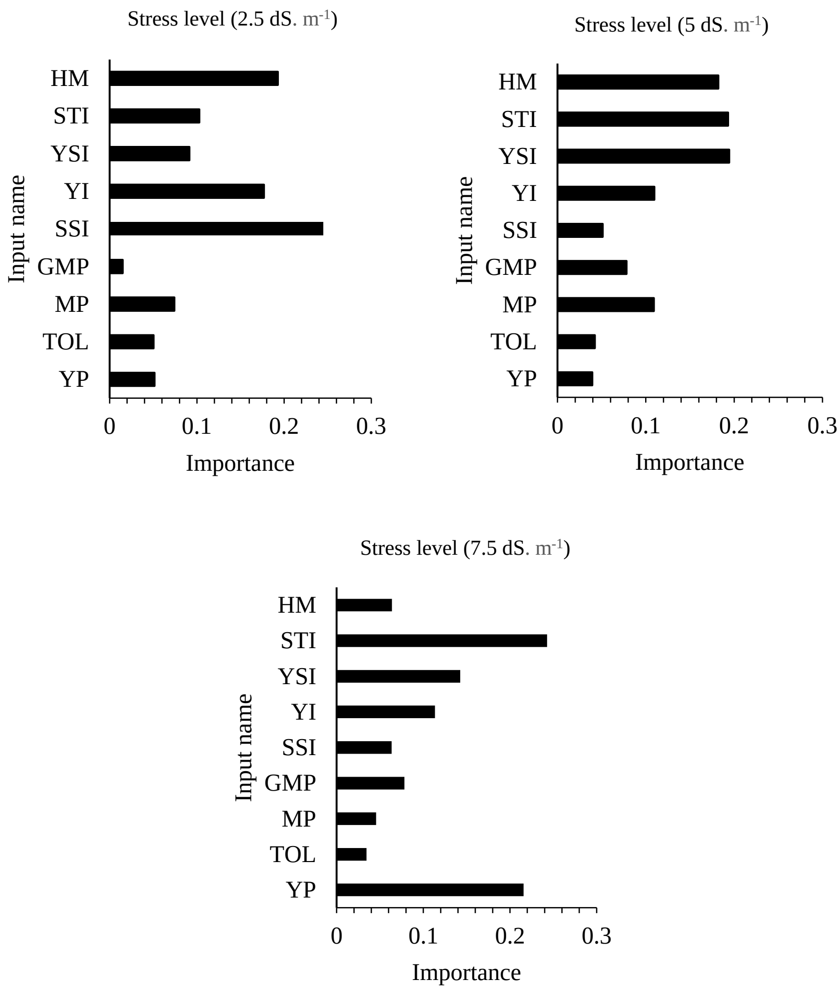

Fig. 6. The relative importance of the effective parameters in determining salt-tolerance indices. [YP: Yield in stress condition; YS: Yield in non-stress condition; TOL: Stress tolerance; MP: Mean productivity; GMP: Geometric mean productivity; SSI: Stress susceptibility index; YI: Yield index; YSI: Yield stability index; STI: Stress tolerance index; HM: Harmonic mean]

Comparing different models

The highest R^2 at different salinity levels was related to the $ANN_{(MLP)}$ model (Table 12).

In addition, the R^2 in $ANN_{(RBF)}$ at different salinity levels was 0.95 (Fig. 7).

Table 12. Performance indices (R^2, RMSE, VAF, MAPE, and RPD) for the models evaluated

Model	Performance indices	Salt stress levels (dS. m^{-1})		
		2.5	**5**	**7.5**
Adaptive neuro fuzzy inference system (ANFIS)	R^2 (Coefficient of determination)	0.85	0.88	0.92
	VAF (Value account for)	86.96	88.46	92.22
	MAPE (Mean absolute percentage error)	3.01	2.27	3.09
	RMSE (Root mean square error)	1.11	0.75	0.50
	RPD (Relative percent difference)	1.65	2.07	2.44
Artificial neural network $_{\text{(Multilayer perceptron)}}$ (ANN $_{(MLP)}$)	R^2 (Coefficient of determination)	0.99	0.99	0.99
	VAF (Value account for)	99.90	99.94	99.91
	MAPE (Mean absolute percentage error)	0.61	0.55	0.56
	RMSE (Root mean square error)	0.07	0.05	0.05
	RPD (Relative percent difference)	23.40	29.83	24.30
Artificial neural network $_{\text{(Radial basis function)}}$ (ANN $_{(RBF)}$)	R^2 (Coefficient of determination)	0.95	0.95	0.95
	VAF (Value account for)	95.37	95.19	95.32
	MAPE (Mean absolute percentage error)	4.86	4.71	5.03
	RMSE (Root mean square error)	0.56	0.48	0.37
	RPD (Relative percent difference)	3.25	3.16	3.23
Genetic algorithm (GA)	R^2 (Coefficient of determination)	0.86	0.85	0.89
	VAF (Value account for)	86.71	86.41	89.40
	MAPE (Mean absolute percentage error)	4.76	4.47	7.31
	RMSE (Root mean square error)	1.10	1.11	0.58
	RPD (Relative percent difference)	1.72	1.71	2.05
Ordinary least squares (OLS)	R^2 (Coefficient of determination)	0.86	0.86	0.91
	VAF (Value account for)	87.40	86.51	91.82
	MAPE (Mean absolute percentage error)	3.89	3.08	3.49
	RMSE (Root mean square error)	1.07	0.82	0.51
	RPD (Relative percent difference)	1.75	1.92	2.38
Principal component regression (PCR)	R^2 (Coefficient of determination)	0.87	0.89	0.92
	VAF (Value account for)	87.36	89.09	92.28
	MAPE (Mean absolute percentage error)	2.96	2.78	3.27
	RMSE (Root mean square error)	1.02	0.73	0.50
	RPD (Relative percent difference)	1.68	2.09	2.45
Partial least squares (PLS)	R^2 (Coefficient of determination)	0.86	0.88	0.92
	VAF (Value account for)	86.97	88.15	92.27
	MAPE (Mean absolute percentage error)	4.34	3.08	3.55
	RMSE (Root mean square error)	1.09	0.76	0.50
	RPD (Relative percent difference)	1.72	2.01	2.45

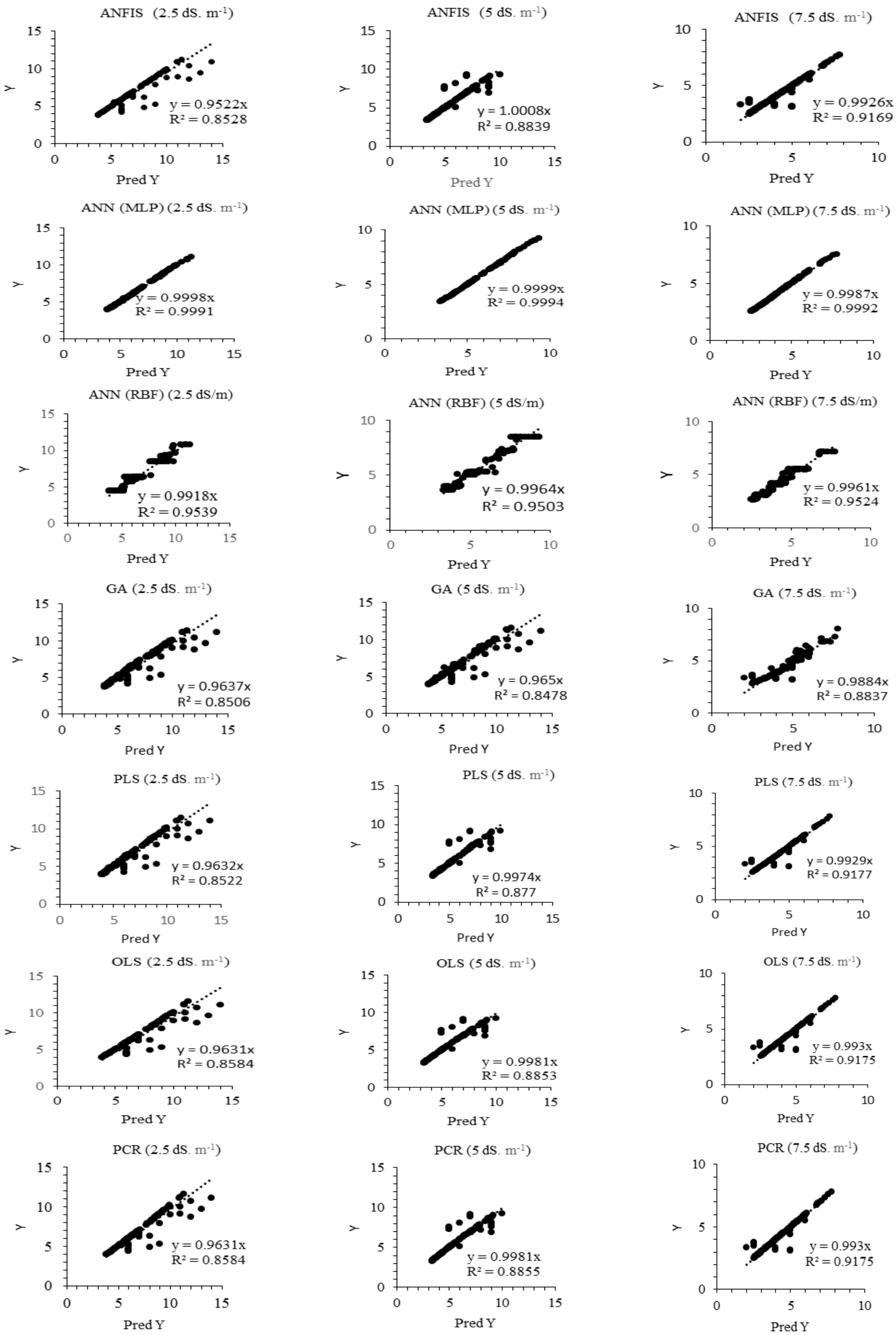

Fig. 7. Variation of estimated values of dry matter against measured data. [ANFIS: Adaptive neuro fuzzy inference system; ANN: Artificial neural network; MLP: Multilayer perceptron; RBF: Radial basis function; GA: Genetic algorithm; OLS: Ordinary least squares; PCR: Principal component regression; PLS: Partial least squares]

The lowest R^2 at 2.5 dS m^{-1} salinity level was related to the ANFIS model. At 5 and 7.5 dS m^{-1} salinity levels, the lowest R^2 was estimated in the GA model. Based on the assessed models, the highest R^2 was found at 7.5 dS m^{-1} salinity level. The amount of RMSE in all models except the genetic model showed a decreasing trend with increasing salinity levels. Furthermore, in the genetic algorithm model, the amount of RMSE had a rising trend at 2.5 to 5 dS m^{-1} salinity levels and reduced its amount at 7.5 dS m^{-1} salinity level. The highest amount of VAF was related to $ANN_{(MLP)}$ model. The VAF rates increased in all evaluated models except for the ANN model with increasing salinity levels. The highest RPD in the PLS and PCR models was obtained as 2.45. The results showed that RPD increases with increasing salinity level. The lowest MAPE was found in the $ANN_{(MLP)}$ model. The $ANN_{(RBF)}$, PLS and GA models showed the highest MAPE. The equations developed by the models tested at different salinity levels are given in Table 13.

Table 13. The equations developed by the models tested at different salt stress levels

Stress level (dS. m^{-1})	Models	Equations
2.5	Partial least squares (PLS)	YS = -0.28+0.21×MP-0.51×SSI+2.17×YI+2.08×YSI+1.01×STI+0.22×HM
	Ordinary least squares (OLS)	YS = 0.09-0.51×MP+0.25×SSI+9.93×YSI+0.44×STI+0.28×HM
	Principal component regression (PCR)	YS = 6.58+0.93× MP +0.08× SSI -0.06× YSI +2.23× STI +0.63× HM
	Genetic algorithm (GA)	Ys = 0.012 -0.236×MP-0.551×SSI+6.221×YSI-0.751×STI+0.646×HM
5	Partial least squares (PLS)	YS = -1.70+0.15×MP+0.15×GMP+3.55×YI+1.74×YSI+0.75×STI+0.16×HM
	Ordinary least squares (OLS)	YS = -0.09+0.03×MP-0.006×GMP-0.78×YI+10.24×YSI-1.03×STI+0.06×HM
	Principal component regression (PCR)	YS = -0.09+7.67×MP-16.24×GMP-0.79×YI+8.76×YSI-1.03×STI+8.75×HM
	Genetic algorithm (GA)	YS =2.729+2.500×MP-1.382×SSI+4.868×YSI+1.682×STI-2.532×HM
7.5	Partial least squares (PLS)	YS = 0.53-0.12×YP+0.21×GMP-0.37×YI+6.55×YSI+0.69×STI
	Ordinary least squares (OLS)	YS = 0.41-0.02×YP-0.04×GMP-0.78×YI+9.2×YSI+0.07×STI
	Principal component regression (PCR)	YS = 0.41-0.02×YP-0.04×GMP-0.78×YI+9.22×YSI+0.07×STI
	Genetic algorithm (GA)	YS =0.099311+0.632×Yp-1.384×GMP+5.753×YI+2.335×YSI+5.629×STI

Discussion

Selection of tolerant ecotype based on stress tolerance indices is very important in agronomy and plant breeding. In our experiment, there were significant differences between different ecotypes based on tolerance indices and this can be helpful for screening the most tolerant ecotypes. Fernandez (1992), based on the response of ecotypes to stress or non-stressed environments, classified ecotypes into four groups. In the group A, genotypes were found to have superior performance in both conditions. In the group B, the genotypes had higher yields only under non-stress conditions. In the group C, genotypes with higher relative yields under stress conditions were placed. In the group D, genotypes were found to have a low yield under normal and stress conditions. This means that TOL, MP, GMP, YSI, STI and HM indices are suitable for isolating ecotypes belonging to group A from B, C and D. These results are in agreement with those reported by Ravari et al. (2015) and Izaddoost et al. (2013). Henfy et al., (2013) have found that GMP, MP, HM and STI indices are suitable for sorghum genotypes. In addition, El-Hendawy et al. (2017) showed that the MP and GMP indices are desirable for selection of genotypes that have high yield under stress and non-stress conditions.

Due to the strong interaction between genotype and environment, the selection is complicated, especially under unpredictable climatic conditions (Romagosa et al, 2013). According to Fernandez (1992), it is the best to identify group A from other groups, because sustainability is higher in genotypes related to this group. Due to the wide variety of soil and water quality in mint production area, ecotypes should be sought, with moderate yields in both saline and normal conditions.

In our experiment, cluster analysis divided ecotypes into different groups based on stress tolerance indices. Ravari et al. (2015) evaluated 41 wheat genotypes based on salt tolerance indices and reported that cluster analysis based on UMGMA method differentiated the genotypes into four groups. On the other hand, principal component analysis plays a decisive role in finding the relationship between stress tolerance indices and the studied ecotypes and has helped us to determine important indices. Hence, our result showed that using the first component results, tolerant ecotypes will be selected based on high-ranking yield performance and tolerance indices such as YP, YS, TOL, MP, GMP, YSI, STI and HM. In this case, ecotypes included E13, E14, E15, E16 and E18 were identified as the tolerant ecotypes with suitable performance under both non-stress and salt-stress conditions. Abraha et al. (2017) evaluated 144 tef (*Eragrostis tef*) genotypes under drought stress. The results of principal component analysis showed that the MP, HM, GMP, and STI indices were identified as the first factor affecting yield in both stress and non-stress conditions.

Sensitivity analysis is one of the strategies that are very important for finding indicators that influence the rate of yield production under stress conditions. Since no study has been conducted in this area so far, our experiment has acceptable performance results under salt-stress conditions. Hence, results of sensitivity analysis showed that STI, YSI, and YI indices were significant in all three stress levels based on their effects on dry matter yield under salinity stress conditions. Hosseini et al. (2017) used the sensitivity analysis to determine the useful parameter on the amount of phosphorus. Moreover, Naroui Rad et al. (2015) used sensitivity analysis to show that flesh diameter and fruit length traits have the most sensitivity to melon fruit yield.

Albeit plant science and other fields have been using intelligent and regression models, this is the first time that multiple models have been used to predict medicinal plant dry matter and have been compared as a group. On the other hand, some preceding efforts to use intelligent or regression models to predict important parameters have been made.

The results of our experiment with different models of artificial intelligence and regression showed that the ANN $_{(MLP)}$ model was the best method for predicting the dry matter yield of mint in salt stress conditions. Hosseini et al. (2017) predicted the amount of phosphorus by intelligent and regression models and found that the ANN and PLS models had higher predictive power. Khaledian et al. (2017) used PLS, OLS and PCR regression models to predict soil erosion. They showed that the PLS model had more efficiency in predicting soil erosion compared with the other models. Minasny et al. (2001) used the ANN to model soil pH and calcium chloride and found that the ANN model predicted better than the linear model. Hosseini et al. (2016) used particle swarm optimization, genetic algorithm and multiple regression methods to predict soil mechanical resistance and found that the intelligent models are better than the regression model.

Conclusion

Researchers use stress tolerance indicators to select the most resistant genotypes. But this has not been done with medicinal herbs so far. On the other hand, the use of intelligence and regression models to predict the performance of dry matter of mint on the basis of stress tolerance index has not been made till now. Therefore, our most important goal was to compare different models for predicting the dry matter performance of different mint ecotypes based on stress-tolerance indices. The results showed that $ANN_{(MLP)}$ model with $R^2 = 0.999$ was the best model for prediction at all salinity levels. The results also showed that ecotypes included E13, E14, E15, E16 and E18 could be used as stress tolerant ecotypes for the future breeding programs. Finally, we conclude that computer software can be very useful in selecting and predicting desired physiological indices and this can be helpful for future projects in plant breeding and physiological programs.

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