OPTIMIZATION OF RED BEAN SEEDS ULTRASONICATION FOR INCREASING GERMINATION AND SEEDLING GROWTH, USING ARTIFICAL NEURAL NETWORK

YOUNESIAN, A.[1*] – GHOLIPOOR, M.[2]

[1]*Young Researchers and Elite Club, Shahrood Branch, Islamic Azad University, Shahrood, Iran*

[2]*Faculty of Agriculture, Department of Agronomy, Shahrood University of Technology, Iran*
e-mail: manouchehr.gholipoor@gmail.com

**Corresponding author*
e-mail: Amir_younesian@yahoo.com

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Abstract. In most cases, there is no positive strong relation (correlation > +0.95) between germination percentage (G) and individual seedling dry weight (W) over treatment levels, which indicates the different response of GW to treatment. In such cases, it is difficult to choose/recommend a treatment level as a best one. Artificial neural network-based optimization (interpolation of treatment levels for possible simultaneous maximum increase in both GW) is the solution to such difficulties. This study aimed at optimizing ultrasonication (pre-soaking duration, temperature and duration of irradiation) of *Phaseolus vulgaris* L. seeds. Treatments were factorial arrangement of 5 pre-soaking durations (2, 4, 6, 8 and 12 hours), 5 irradiation durations (0, 3, 6, 9 and 12 minutes), and 4 irradiation temperatures (temperature of ultrasound device:17, 22, 27, and 32 ^{o}C). The results indicated that the structure 3:4:4:2 of neural network appeared to be appropriate for predicting GW responses. This structure showed higher R^2 in both learning and test phases, when the model was based on Secant Hyperbolic function. The optimized combination of treatments was irradiation temperature of 26.99 ^{o}C, irradiation duration of 5.91 minutes, and pre-soaking duration of 3.20 hours.
Keywords: *pre-soaking duration, irradiation temperature, irradiation duration, seedling dry weight, prediction.*

Introduction

Several methods have been used for seed pre-sowing treatment in order to increase the germination and uniformity i.e. gibberellic acid, sulphuric acid, and hot water treatment (Machikowa et al., 2013). However, seeds treated by these methods have to be rapidly grown and cannot be storaged. Therefore, the methods that increase seed germination but do not affect their viability during the storage are much needed. Applications of ultrasound have been used to induce faster and greater seed germination of the Norway spruce, barley, orchids and other crops. It has been reported that the optimum condition of treated seeds lead to uniform and rapid germination (Machikowa et al., 2013). Ultrasonication not only promotes germination rate (Gordon et al., 1963; Shimomura, 1990; Keshvari et al., 2008; Yaldagard et al., 2008), but also accelerates water uptake and seed coat permeability via creating temperature and mechanical effects on cell membrane and hence promotes germination (Yıldırım and Mehmet, 2015; Gavrilo et al., 1996).

Ultrasonic waves have various applications ranging from seed treatment and elimination of pests and pathogens to genetic engineering and gene transfer (Yaldagard et al., 2008). The first research on the field of biological effects of ultrasound was reported by Harvey and Loomis (1928). In a research conducted on seed germination of

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Assa foetida L., the 0, 4, 8 and 12 minutes ultrasonication resulted in germination rates of 5, 35, 57.75 and 62.5 day^{-1}, respectively; in this study, the 4-minute irradiation was selected as the best treatment (Abdali et al., 2010). Machikowa et al. (2013) reported that seed irradiation with ultrasound waves enhances seedling vigor; this positive effect of ultrasound on seed germination results from change in cellulosic membrane and enhancement of nutrient uptake and transfer to inside of seed and growing seedling. Faryabi et al. (2008) studied effects of ultrasound waves on physiological and morphological processes of capsicum pepper (*Capsicum annuum*) and radish (*Rhaphanus sativus*); in their experiment, the seeds were treated under ultrasound waves of 42 KHz for 0, 4, 6 and 8 minutes at 25 ^{o}C under cool light; results indicated that the best treatment for radish was sonication for 6 minutes.

It should be noted that the amplitude of ultrasound plays critical role in activation/inactivation of enzymes. Many reports confirm enhancement of enzymatic activity of free enzymes under slight radiation of ultrasound; for example, activity of alpha-chymotrypsin on casein is enhanced under slight amplitude of ultrasound; while the activity of this enzyme is reduced at higher amplitudes (Ishimori et al., 1981).

Artificial neural network is a data analysis system inspired from human brain that analyzes the data using a large number of small processors. These processors are arranged as a consistent network and act in parallel to solve a problem. In such networks, a data structure is designed to act like a neuron. An artificial neuron is system which consists of many inputs and one or a few outputs. The network includes layers and weights components. Network behavior depends on the interrelations among the members. In general, there are three types of neuron layers in neural networks:

- Input layer (regressor): reception of raw material (R_1, …, R_n) entering the network.
- Hidden layer(s): performance of these layers is determined by inputs and the weights of their relations (Z_1, …, Z_2). The weights between input and hidden units determine when a hidden unit should be active.
- Output layer: function of output layer depends on function of hidden unit and the weight of relation between hidden and output layer (Y_1, …, Y_n).

Neural networks have many applications such as prediction of continuous variables such as soil moisture (Chang and Islam, 2000), sampling (Zhang and Barrion, 2006), estimation of grain biomass and yield (Drummond et al., 2003). Using neural network, Gholipoor et al. (2013) optimized the traits affecting barley seed and suggested that genetic improvement of barley seeds based on the optimized seeds can significantly improve seed yield. By optimization of mineral concentration in sugar beet tuber using artificial neural network, the optimal concentration of calcium, magnesium, nitrogen, potassium and sodium was estimated as 0.37%, 0.35%, 0.97%, 4.67 mili equivalent/100 g, and 0.33%, respectively. Under optimized concentration of minerals, potential yield of tuber and sugar was enhanced by about 17% (Gholipoor, 2012).

To the best of our knowledge, there has been no report about possible interaction of pre-soaking duration and ultrasonication temperature with ultrasonication duration on seedling growth and germination percentage of red bean. Regarding these issues, the main objective of this study was to find the best ultrasonication duration, pre-soaking duration and ultrasonication temperature to have highest germination percentage and seedling growth.

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Due to the fact that in most cases, there is no positive strong relation (correlation > +0.95) between germination percentage and individual seedling dry weight over treatment levels (Golipoor et al., not published), these traits tend to have different response to a treatment. In such cases, it is difficult to choose/recommend a treatment level as a best one. Artificial neural network-based optimization (interpolation of treatment levels for possible simultaneous maximum increase in both germination percentage and individual seedling dry weight), which was used here, is the solution to such difficulties.

Materials and Methods

Laboratory experiment

An experiment, as completely randomized design with three replications, was carried out on red bean (*Phaseolus vulgaris* L.) seeds in Seed Technology Center of Shahrood University of Technology in 2015. Treatments were factorial arrangement of five pre-soaking durations (2, 4, 6, 8 and 12 hours), five ultrasonication durations {0 (control), 3, 6, 9 and 12 minutes}, and four ultrasonication temperatures (temperature of ultrasound device: 17, 22, 27, and 32 ^{o}C). The ultrasonic bath (digital ultrasonic, Model 4820-CD) with constant frequency of 24 kHz of ultrasonic waves was used for irradiating the seeds.

Twenty five seeds were selected for each petri dish. The germinated seedlings were counted on daily basis until it was found no change in number of germinated seedlings for two consecutive days. The germinated seedlings were multiplied by four to get the germination percent. The seedlings were separated form seeds and dried in oven for 48 hours at 70 ^{o}C. Then they were weighed. The individual seedling dry weight and germination percentage were subjected to analysis of variance, using the SAS software (version 9.1).

Optimization of regressors using artificial neural network

Data of input (regressors or independent variables; pre-soaking duration, ultrasonication duration, and ultrasonication temperature) and output variables (dependent variables; individual seedling dry weight and germination percentage) were first arranged consecutively and divided into two learning (70%) and test (30%) parts. Then, they were standardized using the following formula (Rohani et al., 2011):

$$Y_i = 0.8 \times \frac{X_i - X_{min}}{X_{max} - X_{min}} + 0.1 \qquad \text{(Eq.1)}$$

where, Y_i represents standardized data, X_i denotes non-standardized data, X_{min} stands for the smallest data and X_{max} denotes the largest data. Using this formula, the input data are placed between 0.1 and 0.9. Perceptron multilayer neural network was used in this research. Using QNET software, back propagation algorithm was used for network learning. Various numbers of hidden layers and following four activation (transfer) functions were tested:

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- Sigmoid function:

$$f\left(\sum W_{ij}X_i\right) = \frac{1}{1+e^{-\left(\sum_{i=1;j=1}^{n} W_{ij}X_i\right)}} \quad \text{(Eq. 2)}$$

- Hyperbolic tangent function:

$$f\left(\sum W_{ij}X_i\right) = \frac{e^{\left(\sum_{i=1;j=1}^{n} W_{ij}X_i\right)} - e^{-\left(\sum_{i=1;j=1}^{n} W_{ij}X_i\right)}}{e^{\left(\sum_{i=1;j=1}^{n} W_{ij}X_i\right)} + e^{-\left(\sum_{i=1;j=1}^{n} W_{ij}X_i\right)}} \quad \text{(Eq. 3)}$$

- Hyperbolic secant function:

$$f\left(\sum W_{ij}X_i\right) = \frac{2e^{\left(\sum_{i=1;j=1}^{n} W_{ij}X_i\right)}}{e^{2\left(\sum_{i=1;j=1}^{n} W_{ij}X_i\right)} + 1} \quad \text{(Eq. 4)}$$

- Gaussian function:

$$f\left(\sum W_{ij}X_i\right) = \frac{1}{\sqrt{2\Pi\sigma}} e^{\frac{-\left[\left(\sum_{i=1;j=1}^{n} W_{ij}X_i\right)-\Pi\right]^2}{2\sigma^2}} \quad \text{(Eq. 5)}$$

where W is weight, X regressor, σ standard error, and Π pi value.

For evaluation of efficacy of perceptron neural network in predicting variation of seedling dry weight and germination percentage, the correlation coefficient (r), mean of absolute error (MAE) (eqaution 6), root-mean-square error (RMSE) (eqaution 7) and relative standard deviation (RSE) (eqaution 8) were used:

$$\text{MAE}=\frac{1}{n}\sum_{i=1}^{n}\left|Y_i - \hat{Y}_i\right| \quad \text{(Eq. 6)}$$

$$\text{RMSE}=\sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(Y_i - \hat{Y}_i\right)^2} \quad \text{(Eq. 7)}$$

$$\text{RSE}=\frac{\sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(Y_i - \hat{Y}_i\right)^2}}{Ybar} \quad \text{(Eq. 8)}$$

where, Y_i stands for observed output, Ybar denotes mean of observed output, $\hat{Y}_i$ is estimated output and Ybar stands for mean of observed output. Optimization of the regressors was performed through following three steps (Gholipoor et al., 2012), using QNET software:

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Step 1

Out of the regressors, a regressor (irradiation temperature) with strong relation with seedling dry weight and germination percentage was put in the model as the output. Seedling dry weight and germination percentage were considered as model inputs. Data were randomly arranged in line and divided in to two learning (70%) and (30%) parts. Different scenarios were tested and the superior model was selected for each output variable. Based on the superior model, irradiation temperatures were estimated. Each of these estimated values can be potentially the optimal temperature of irradiation for achieving maximum seedling dry weight and germination percentage. The temperature values within tested temperature range were selected (*Table 1*).

Step 2

Using original and estimated data in previous step, other regressors were individually put in the model as output. Model inputs include seedling dry weight, germination percentage and irradiation temperature. Like previous step, data were divided into two groups, different scenarios were tested and superior model was selected. Using this superior model, output was estimated. The values within the tested values range were selected (*Table 1*).

Step 3

For prediction of seedling dry weight and germination percentage using estimated regressors, the neural network model developed earlier was used. The predicted values of seedling dry weight and germination percentage were presented in *Table 1*.

Table 1. *The values of input layer and output layer obtained during 3 steps of optimization*

Irradiation temperature (^oC)	**Pre-soaking duration (hour)**	**Irradiation duration (minute)**	**Seedling dry weight (mg.seedling^{-1})**	**Germination percentage**
17.09954	2.10646	0.24924	0.21596	87.60438
17.02344	5.86315	0.32141	0.28215	85.12283
27.06161	7.84034	5.74945	0.26376	88.31532
26.40762	3.82008	4.06223	0.30046	85.75182
27.31862	5.57029	6.15325	0.27945	84.88587
26.47268	5.96725	5.54694	0.28916	87.97795
31.98362	9.14480	11.98689	0.32757	87.49119
16.67356	12.13849	8.54124	0.32846	83.72129
32.13457	3.00300	11.52284	0.25481	98.56429
26.52020	12.56619	6.83293	0.27769	96.19994
30.24704	10.97450	3.64731	0.34973	93.71431
17.30544	12.35777	0.02311	0.37052	78.55254
29.81815	3.94204	1.51304	0.24119	90.53204
21.94637	11.87978	6.04494	0.36646	88.20433
26.65126	6.11294	9.39194	0.35170	94.77507
26.99524	3.20238	5.91580	0.48892	100.00000
17.05237	3.03896	11.89409	0.22875	78.82457

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26.44926	7.01220	5.37727	0.27758	90.83411
16.87708	8.02893	6.13390	0.35306	88.75356
28.66749	6.01602	10.52825	0.32855	91.69682
26.89883	2.19398	11.62910	0.26829	93.83383
29.31988	1.93755	0.00086	0.25591	84.39989
32.09550	2.70404	9.50710	0.28553	96.12952
25.93219	6.18928	0.17911	0.18947	80.17261
22.18381	1.48999	3.35011	0.31006	97.18890
26.45018	7.03788	5.35032	0.27777	90.95387
21.63031	8.07121	11.65102	0.28035	89.79551
17.17671	12.53354	6.24890	0.31022	83.34408
21.99522	6.00882	4.94837	0.27886	97.32630
22.16796	7.50277	0.30533	0.27186	88.85596
26.79841	3.27539	6.09689	0.31378	93.06115
26.54209	7.88851	7.02443	0.19549	80.10416
31.83300	12.26652	3.68888	0.36477	95.79995
26.54753	6.31874	7.11125	0.21100	80.38619
31.96131	10.17290	11.87890	0.31806	86.72826
22.01154	8.00452	8.61893	0.36592	93.77309
26.90156	2.60097	5.81457	0.31348	95.63342

Results and Discussion

The substantial differences in treatments produced a wide range of variation in the values of the output layer (*Table 2*). For instance, the maximum value of seedling dry weight was almost twice of minimum value; such variations are necessary for successful learning and predicting processes of neural network. Specially, when there is no strong relation ($r>+0.95$) between output variables; the relation of seedling dry weight with germination percentage was 0.34 which confirms the different response of these traits to treatments.

The result of analysis of variance indicated that in addition to simple effects, the double and triple interactive effects of factors pre-soaking duration, temperature regime, and irradiation duration were significant on both seedling dry weight and germination percentage traits (data not shown). This shows that the effect of one factor on these traits tends to be changed with changing the level of other two factors. Such interactive effects, on one hand, and not significant relation between traits, on the other hand, indicate the complicate relation between regressors and output variables. Neural network-based optimization is capable of solving such multi-response complicate problems.

The performance of the MLP tended to improve as the number of hidden neurons increased. However, too many neurons in the hidden layer caused over-fitting problems. This situation allowed good network learning and data memorization but also produced a lack of any ability to generalize. However, the network was unable to learn if a small number of neurons were used in the hidden layer. Usually the number of layers and neurons nodes of hidden layer (s) is typically determined by trial-and-error. The best structure of neural network was 3:4:4:2.

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Statistical indices were used for evaluation of neural network models for (1) different activation functions, (2) number of hidden layers, and (3) number of neurons in each hidden layer. Combination of these factors can generate more than 50 scenarios and presenting statistical indices for all of these scenarios provides an unnecessary and bulky table. Therefore, after selection of the best number of hidden layer and neuron number in the hidden layer, only statistical indices for activation functions are presented (*Table 2*). According to these indices, higher accuracy of a neural network is obtained when the model not only has higher coefficient of determination, but also has lower absolute error mean, lower relative standard deviation, and lower root-mean-square error. This is the case for hyperbolic tangent activation function as is evident in *Table 3* and *Figs. 1 and 2*.

Table 2. *Statistical properties of input and output layers of neural network model*

Input+output	Range	Mean	Maximum	minimum
Irradiation temperature (^{o}C)	15.0000	24.5758	32.0000	17.0000
Pre-soaking duration (hour)	10.0000	6.3434	12.0000	2.0000
Irradiation duration (minute)	12.0000	6.0303	12.0000	0.0000
Seedling dry weight (mg. seedling^{-1})	0.2601	0.2966	0.4056	0.1905
Germination percentage	23.3333	90.2862	100.0000	76.6667

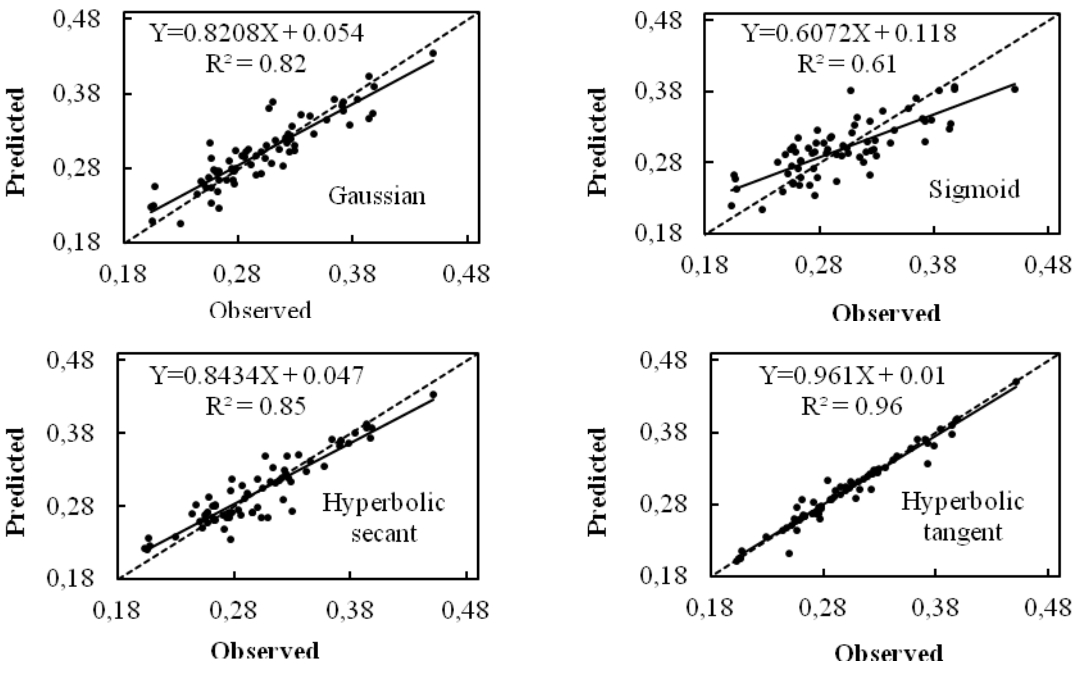

Figure 1. *Sensitivity analysis of neural network using four activation functions for prediction of seedling dry weight of red bean in learning phase*

Optimization results indicated that the highest germination percentage and seedling growth optimized by neural network were 100% and 0.48892 g, respectively (*Table 1*), which is about 19% higher than the observed maximum dry weight of seedling. These values were obtained under treatment with irradiation temperature of 26.99 ^{o}C,

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irradiation duration of 5.91 minutes and pre-soaking for 3.20 hours. This combination resulting from neural network is different from treatment combination in routine analyses (temperature of 32 °C, pre-soaking for 12 hours and irradiation duration of 12 minutes).

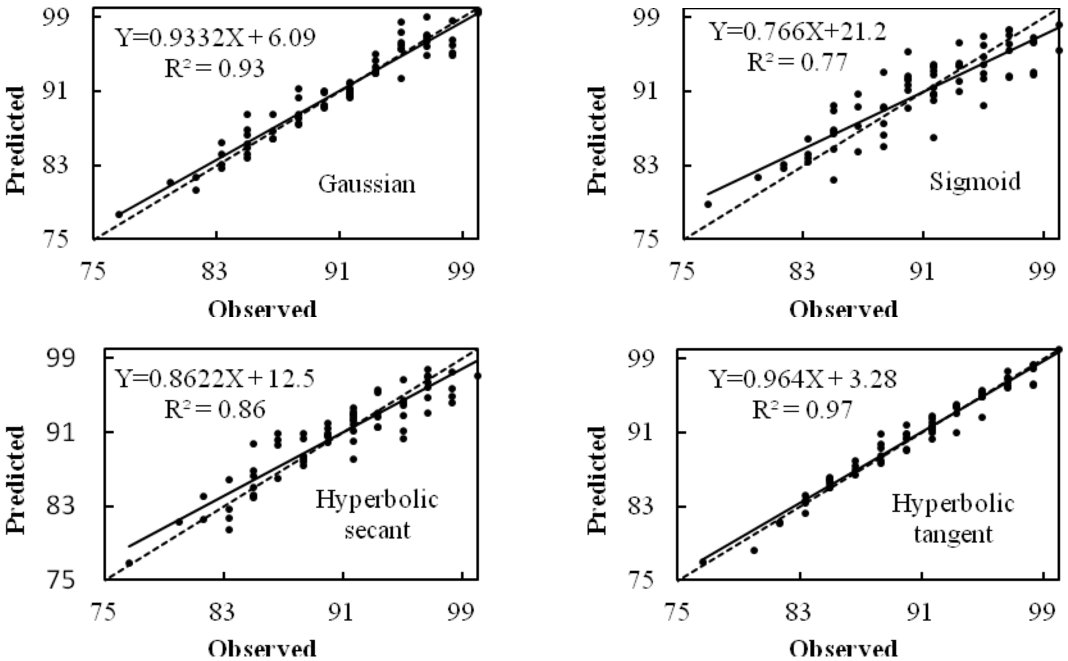

Figure 2. *Sensitivity analysis of neural network using four activation functions for prediction of germination percentage of red bean in learning phase*

The best irradiation level obtained here for *Phaseolus vulgaris* L. , 5.91 minutes, is similar to 6 minues for *Capsicum annuum* L. and *Rhaphanus sativus* L. (Faryabi et al., 2008), but inconsistent with 4 minutes for *Assa foetida* L. (Abdali et al., 2010). Such differences or similarities imply the expectedly direct relation between hardness of seed coat and exposure duration; in another words, the exposure duration is highly dependent on texture of seed covers. This is because of the fact that the machanical impact of ultrasound is higher than other priming methods like hydro priming, hormon priming, and magnetic field (Majd et al., 2010), as ultrasonication could fragment the seed shell and produce larger porosity on the surface of seeds by captivation of ultrasound (shock waves). Shell fragmentation and enlargement of the pore size of seeds lead to more water retention capacity in dry grains and result in better hydration (Yaldagard et al., 2008).

Table 3. *Statistical indices of various transfer functions*

Transfer function	Dependent variable	RMSE	MAE	RSE	r
Gaussian	Seedling dry weight	0.00567	0.01645	0.01889	0.90550
	Germination percentage	0.04410	1.06378	0.00049	0.96424
Sigmoid	Seedling dry weight	0.00788	0.02642	0.02626	0.78187
	Germination percentage	0.14344	2.03956	0.00158	0.87762
Hyperbolic tangent	Seedling dry weight	0.00191	0.00570	0.00636	0.98012
	Germination percentage	0.11125	0.67271	0.00123	0.98508
Hyperbolic secant	Seedling dry weight	0.00013	0.01521	0.00045	0.92348
	Germination percentage	0.04628	1.55737	0.00051	0.92583

Regarding contribution of regressors in explaining variation of seedling dry weight and germination percentage, irradiation temperature, irradiation duration and pre-soaking duration have the higher priority, respectively; however their difference was not considerable (*Fig. 3*). This result indicates importance of all the three factors and implies that ignoring one factor may result in unfavorable result. This is an exact and unique property of neural network for optimization (Kashi et al., 2013; Huang et al., 2010; Green et al., 2007; Park et al., 2005; Kaul et al., 2005). There is no published report about relative importance of these factors. Regarding different response of plant species to irradiation it is necessary to study impressionability of other species from these factors.

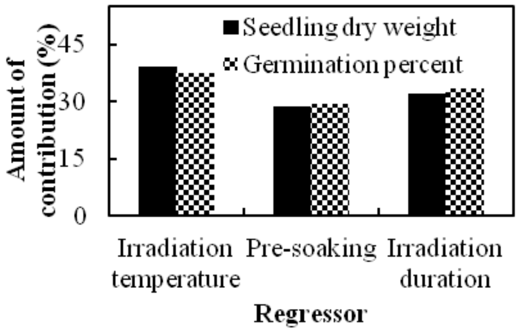

Figure 3. *Contribution of regressors in explaining variation of seedling dry weight and germination percentage*

If ultrasonication is used at optimal conditions (temperature and pre-soaking duration), it will provide positive effects. Irradiation effects result from cavitation phenomenon which causes alteration in cell membrane (Bommannan et al., 1992). One of the reasons for positive effect of ultrasonication on seedling dry weight might be due to reduction in soaking time, as it has been reported for chickpeas (Yildirim et al., 2010), sorghum (Patero and Augusto, 2014) and navy beans (Ghafoor et al., 2015). This improvement has been attributed to a greater reduction of internal resistance than external resistance (Cunningham et al., 2008), as well as possible changes in microstructure by cavitation (micro-channel formation) and/or the so called “sponge effect”, causing inertial flow (Patero and Augusto, 2014). The enhanced movement of liquid medium, increase of mass transfer among the organelles within cells and enhanced rate of biochemical reactions are also regarded as the positive effects of irradiation (Bar, 1998).

Conclusion

The results indicated that seedling dry weight and germination percentage tend to differ in terms of response to treatment level (combination of temperature regime, pre-soaking and irradiation duration), as their correlation was not significant. Therefore, selecting each level of treatment does not necessarily increase both traits. Therefore, interpolation of treatment level for possible simultaneous increase in both seedling dry weight and germination percentage, say optimization, was carried out to solve the problems. The optimized values of treatment levels (ultrasonication

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components) were temperature of 26.99 ^{o}C, irradiation duration of 5.91 minutes and pre-soaking for 3.20 hours. This procedure could be used in all agricultural researches in which the multi-response investigations, like response of grain yield and grain protein content, are common.

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