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Research Paper

Assessing *in vitro* germination and seedling growth of tomato (*Solanum lycopersicum* L.) cv KC-1 in response to polyethylene glycol-induced water stress

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Abstract: Polyethylene glycol (PEG) is a high molecular weight osmoticum, which can be used to induce water stress artificially in a nutrient medium. An experiment was conducted with four concentrations of polyethylene glycol namely 30 g/L, 60 g/L, 90 g/L with a control to study the effect of PEG on seed germination and seedling performance of tomato *cv*. KC-1. The experiment was laid out on a complete randomized design (CRD) with six replicates. Data was analyzed using the regression and Pearson correlation analysis. The PEG concentrations fortified to the culture media had significant negative relationships ($p<0.0001$) for all estimated parameters such as shoot length, root length, fresh weight, dry weight, chlorophyll $_a$, chlorophyll $_b$, total chlorophyll content of the *in vitro* grown tomato seedlings. The MS media fortified with 0 mg/L PEG treatment (control) exhibited better seed germination percentage (87.8%) and seedling performance, while the minimum value of seed germination (21.9%) was noted under 90 g/L PEG treatment at 20 days after culture. In control PEG treatment, high values were noted for shoot length (2.88 cm), root length (1.67 cm), fresh weight (60.37 mg), dry weight (8.93 mg), chlorophyll a (1.38 mg/g), chlorophyll b (0.48 mg/g), total chlorophyll content (1.86 mg/g) after 4 weeks of culture. The decreased in seed germination and seedling growth rate probably due to lower the water potential of the culture media by supplement with the PEG into the MS medium fortified with 2 mg/L BAP. *In vitro* germination and seedling growth were considerably reduced on 60 g/L and 90 g/L PEG concentrations.

Keywords: Germination, *In Vitro* Seedling, PEG, Tomato, Water Stress

Introduction

Tomato (*Solanum lycopersicum* L.) is the most popular vegetable crop in the world (Bhatia *et al*., 2005). It is a model species to introduce economically important genes into dicotyledonous crop plants (Wing *et al*., 1994). Tomato fruit is considerably used to prepare vegetarian and non-vegetarian foods. It contains vitamins and antioxidant flavonoids like lycopene, which may be beneficial for human health (Singh *et al*., 2014). Tomato is a water-demanding crop. Extreme dry weather conditions negatively affect seed germination and subsequent seedling growth and hence boosting cell elongation sensitivity to damages induced under stressed conditions (Delachiave and Pinho, 2003; Hamayun *et al*., 2010).

Under water stress, availability of water and nutrients is decreased in the root zone of plant for their growth and development. Water stress can significantly reduce the yields of most major crops (Bray *et al*., 2000; Flowers *et al*., 2000). Genotype for drought tolerance has been developed by the crossing of cultivated tomato with drought tolerant lines. There are various ways to identify drought tolerant genotypes (George *et al*., 2013). Polyethylene glycol (PEG) has been used to stimulate water stress in seedlings under *in vitro* conditions and it decreases water potential of culture medium (Hassan *et al.,* 2004). Screening using PEG under *in vitro* culture technique is one of the ways to choose the best desirable genotypes to study on water scarcity on seed germination indices (Kocheva *et al*., 2003).

Molecules of PEG 6000 are small enough to change the osmotic potential and tomato has been recognized for better growth under PEG stimulated water stress (Bressan *et al*., 2003). PEG assay was done to screen the level of drought tolerance of tomato for the plant breeding techniques. Hassan *et al*. (2004) stated that the PEG acts as an osmoticum which reduces the water potential of culture medium. In this study, seed germination and seedling growth was assessed under different water stress conditions by using the PEG as osmotic inducer

Materials and Methods

This study was conducted to investigate the effects of polyethylene glycol (PEG) on seedling performance of tomato at the Tissue Culture Laboratory, Department of Crop Science, Eastern University of Sri Lanka in 2018. The experiment was laid out in a complete randomized design and replicated six times. Tomato (*Solanum lycopersicum* L) seeds of KC-1 variety were obtained from Horticultural Crop Research and Development Institute, Gannoruwa, Sri Lanka.

<u>Seed sterilization</u>

Tomato seeds were washed with the distilled water to remove the dirty materials and those were surface sterilized by spraying 70% alcohol for 1 min, and then sterilized in 20% (v/v) Clorox™ for 20 min. Finally, the seeds were washed with the sterilized distilled water for three times to remove the excess Clorox from the seeds. The sterilized seeds were placed inside the petri-dish containing sterilized filter paper for few minutes to remove the moisture from those seeds. They were placed under the sterilized conditions and were rapidly being used for the inoculation.

<u>*In vitro* Seed culture</u>

The sterilized tomato seeds were cultured on the MS basal media supplemented with the 2 mg/L BAP and different concentrations of polyethylene glycol (PEG), such as 0 (as control), 30 g/L, 60 g/L and 90 g/L. All the cultures were incubated under white fluorescent light at temperature of 25±0.5 °C in photoperiod of 16 hrs light and 8 hrs dark. Light intensity of 50 $\mu molm^{-2}s^{-1}$ and 70% humidity were maintained inside the culture room.

<u>Seed germination</u>

Germination of seeds was recorded from the days of seeds sown and observations were continued until four weeks of seeding. Seed germination and seedling growth under water stress were frequently observed. Seeds, which were placed on the different MS media, were observed and numbers of germinated seeds were recorded. Counting was continued daily and final counting was considered as final germination percentage.

<u>Seedling growth</u>

At 4 weeks of seeding, shoot and root length, fresh and dry weights of shoots and roots were recorded. In each treatment, ten seedlings from each replicate were used to take the readings. Length of shoots and roots were recorded in centimetre (cm) by using the graduated ruler. After taking the fresh weight of shoots and roots those were put into paper bags separately with proper labelling for the identification. Then those were placed in oven at 60 °C for oven drying until it reached constant weight. After complete drying, dried shoots and roots were weighed by electrical balance data was recorded.

<u>Chlorophyll content</u>

One hundred milligrams (100 mg) of leaves from the *in vitro* grown tomato seedling were collected after four weeks of culture on MS medium supplemented with the different concentrations of

PEG to determine the chlorophyll content. The collected leaves were then placed in a 25 ml glass vial, added with 10 ml of 80% acetone and blended with a homogenizer. Extract was collected into a test tube. The chlorophyll contents were measured using the UV spectrophotometer at 663 nm and 645 nm wavelengths. Chlorophyll a, chlorophyll b, and total chlorophyll contents were calculated using Equations 1, 2 and 3 (Arnon, 1949);

$$\text{Chlorophyll a (mg/g)} = (12.7*A_{663} - 2.69*A_{645}) \quad \ldots\ldots \quad \text{Equation 1}$$
$$\text{Chlorophyll b (mg/g)} = (22.9*A_{645} - 4.68*A_{663}) \quad \ldots\ldots \quad \text{Equation 2}$$
$$\text{Total chlorophyll (mg/g)} = (20.2\,A_{645} + 8.02A_{663}) \quad \ldots\ldots \quad \text{Equation 3}$$

where, A= Absorbance at specific wavelengths. The data obtained from the experiment were subjected to Regression analysis and Pearson correlation using SAS 9.1.3 portable version

Results and Discussion

<u>Effect of water stress on seed germination</u>

The results showed that seed germination % of tomato gradually decreased with increasing PEG concentrations from 0 g/L to 90 g/L (Figure 1). Higher germination percentage (87.8%) was recorded in the control treatment (0 g/L) followed by 30 g/L of PEG added to the culture medium at 20 days after culture. Khodarahmpour (2011) reported that increasing PEG concentration in the culture medium decrease the seed germination rate. Kulkarni and Desphpande (2007) and Aazami *et al.* (2010) also reported that PEG reduces the germination percentage. PEG reduces the water potential of the culture medium and inadequate water content cause to lower the seed germination (Govindaraj *et al.*, 2010).

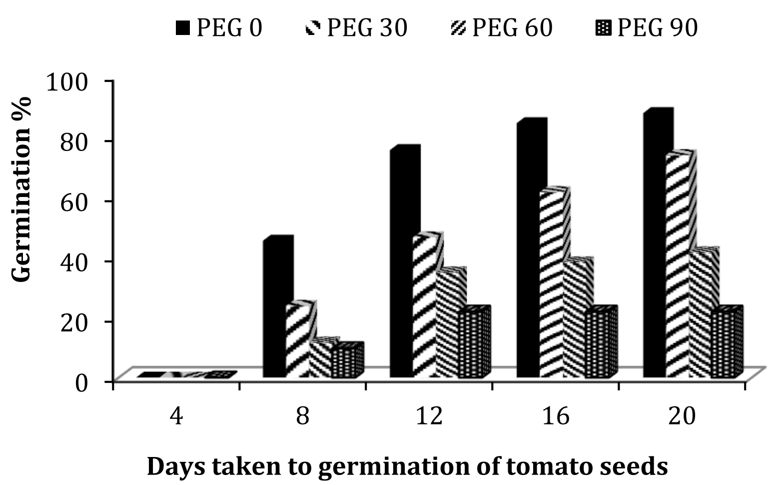

Figure 1: Effect of different concentrations of PEG (g/L) on seed germination % after seed culture.

At 20 days after seed culture, there was a significant relationship between different concentration of PEG and seed germination percentage ($p \leq 0.0001$). A negative regression coefficient implied that when the PEG concentration increased the germination % had a tendency to decrease. The fitted line plot shows the relationship between a PEG concentration and the germination percentage of the cultured seeds (Figure 2). Almansouri *et al.* (2001) reported that sufficient amount of water is an essential for the action of initiation of seed germination. Seed germination is the most perceptive stage in the life cycles of plants (Ahmad *et al.*, 2009).

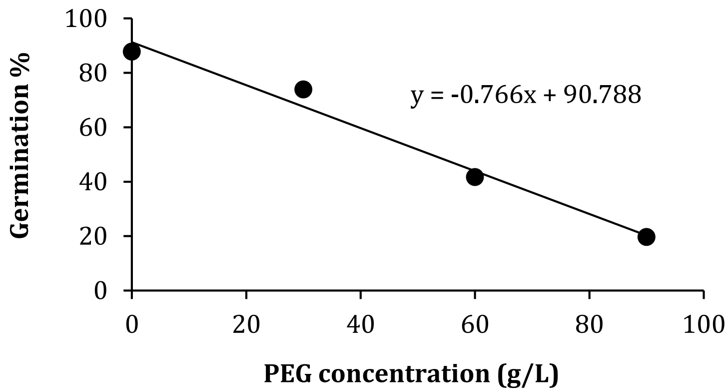

Figure 2: Seed germination % at 20 days after culture on different concentrations of PEG (r=0.879).

Seed germination percentage was 87.8% and 73.9% at 20 days in control and 30 g/L PEG level respectively. None of the treated seeds achieved 100% germination within 20 days even in the control treatments of PEG. Application of 0 g/L and 30 g/L of PEG did not affect the germination and seeds were germinated quickly in these media as compared to other media. Osmotic pressure which is formed by the different PEG levels is caused to lower the water potential of cells that is necessary for the seed germination. Lowering osmotic potential by increasing PEG caused reduction in seed germination percentage and the decrease in seed germination was proportional to the increasing concentration of PEG 6000. This obtained result on seed germination showed that water stress of tomato seed was influenced by the different PEG concentrations and time period which exposure to PEG. Hartmann *et al*. (2005) and also Van den Berg and Zeng (2006) also reported that water stress due to drought is one of the most important abiotic factors that affect the seed germination and seeding growth. Turk *et al*. (2004) stated that water stress during germination phase delayed or decreased germination process.

Effect of water stress on shoot length in seedling

Effect of different PEG solutions on shoot length of seedlings at 4 weeks after culture is presented in Figure 3. Results showed a significant effect ($P \leq 0.0001$) on shoot length of tomato with different concentration of PEG solutions. According to model, coefficient value (-0.027) indicated how much mean of the shoot length decreased given a one-unit change in PEG concentration. Reduction of shoot length was increased when increased the water stress levels gradually. Kulkarni and Deshpande (2007) noted considerable decrease in shoot length of tomato with the increasing PEG concentrations. Aazami *et al.* (2010) also reported that reduction of growth rate in tomato cultivars was due to varying PEG induced drought stress.

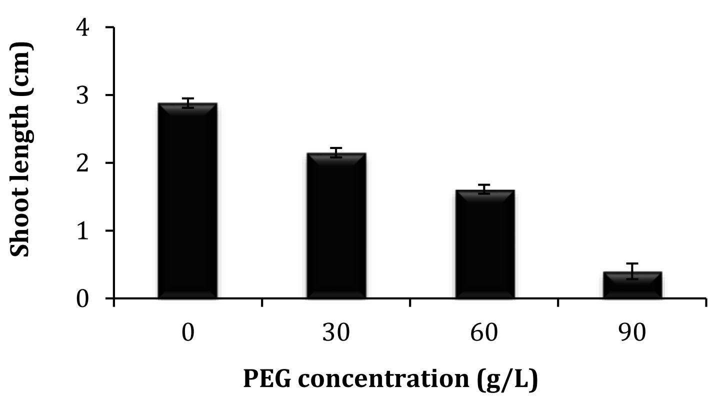

Figure 3: Shoot length of tomato seedlings with different PEG concentrations after 4 weeks of culture (r = 0.964). Vertical lines are the standard error of the means.

Effect of water stress on root length in seedling

Results showed the increasing the levels of water stress in the culture media were influenced to decline the root length of *in vitro* grown tomato seedling (Figure 4). The decrease in the growth of root was more marked than the shoot growth. Jajarmi *et al.* (2009) noted the remarkable reduce in root length with increasing PEG concentrations. These findings are in conformity with Nayar and Gupta (2006) in various plant species of wheat and maize (Nayar and Gupta, 2006). Ghafoor (2013) stated that roots are the primarily affected plant part under drought conditions than any other parts. Kulkarni and Deshpande (2007) reported that early and rapid elongation of roots is a key attribute of drought tolerance. Misra and Dwivedi (2004) and Abida *et al.* (2013) also reported that roots suffer first from exposure to drought followed by their associated plant parts.

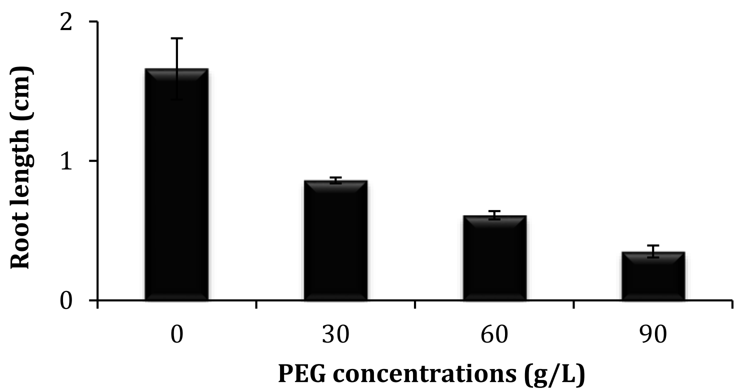

Figure 4: Root length of tomato seedlings with different PEG concentrations after 4 weeks of culture. Vertical lines are the standard error of the means.

Regression analysis was determined increasing the PEG solutions associated with decreasing the root length of seedlings. Regression coefficient revealed the negative relationship between PEG concentration and root length (R^2 = -0.014). Reduction in growth rate with increasing osmatic stress was stated by Raheem *et al.* (2007), Aazami *et al.* (2010) and Hamayun *et al.* (2010). The decline root and shoot growth may be due to toxic effects of the higher level of PEG concentration. Shoot and root growth on different PEG treatments after 4 weeks of culture is shown in Figure 5.

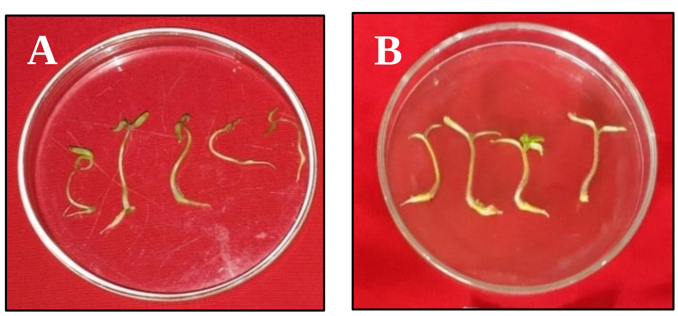

Figure 5: Seedling formation (A-B) at 4 weeks of culture under 0-30 g/l concentrations of PEG treatments

Effect of water stress on fresh weight of seedling

In the regression model, the coefficient showed that there was the negative association between PEG concentration and fresh weight of seedling. The association was statistically significant ($p<0.0001$; Figure 6). Highest value of fresh weight was indicated in the control treatment of PEG with an average 60.36 mg followed by 33.15 mg for the 30 g/L PEG treatment and considerably lowest value was recorded in 90 g/L PEG with an average of 8.60

mg. When increasing the PEG concentration levels, fresh weight of tomato seedling was gradually reduced. Similar finding was reported by Nahar and Gretzmacher (2002) who stated that seedling biomass was affected by PEG solution in tomato.

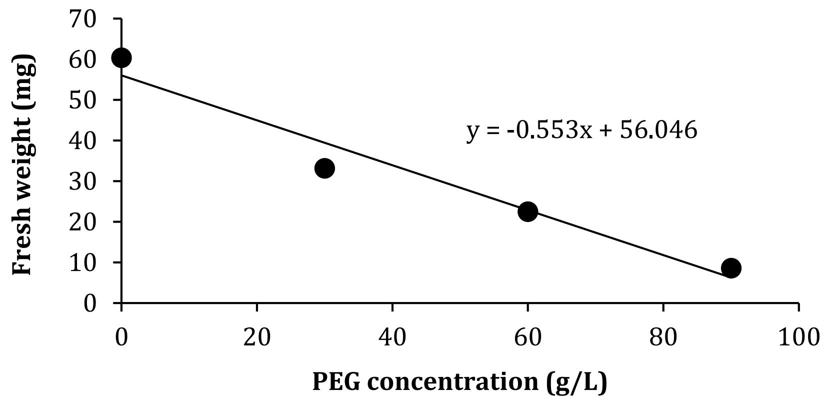

Figure 6: Relationship between different PEG concentrations and fresh weight of tomato seedlings after 4 weeks of culture (r =-0.945)

Effect of water stress on dry weight of seedling

Highest value of dry weight was recorded in the control treatment (0 g/L) of PEG with an average 8.93 mg followed by 2.96 mg for the 30 g/L PEG treatment. Lowest value was noted in 90 g/L PEG with an average of 2.96 mg (Figure 7). Seedling fresh weight also showed a similar trend and it decreased with increasing PEG concentration. Similar results in dry weight of root was reported by Anaytullah and Yadav (2007) in rice cultivars. In regression analysis, there was a significant relationship between PEG concentration and seedling dry weight.

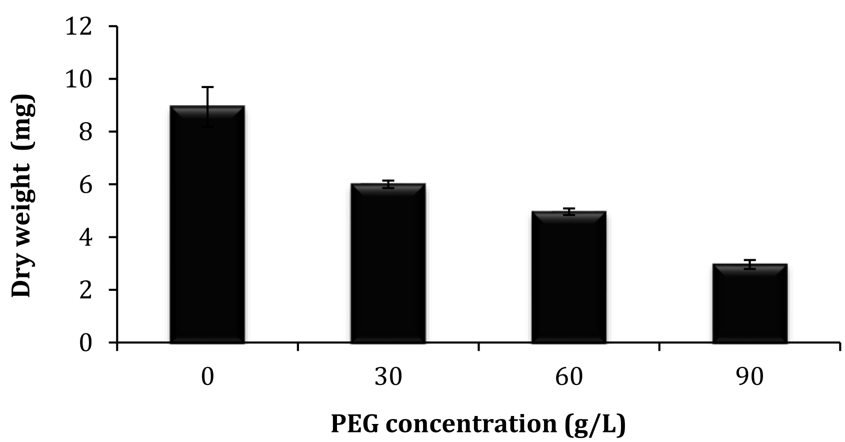

Figure 7: The dry weight of tomato seedlings with different PEG concentrations after 4 weeks of culture. (r = -0.907). Vertical lines indicates the standard error of the means.

Effect of water stress on chlorophyll a, b, and total chlorophyll content of leaves

Chlorophyll a content of seedling was measured in the culture media supplemented with the different concentrations of PEG. Maximum chlorophyll a content was found as 1.38 mg/g at control treatment followed by 1.07 mg/g at 30 g/L PEG treatment (Figure 8). The minimum chlorophyll a content was 0.33 mg/g at 60 g/L PEG treatment among the 0-60 g/L treatments. Reduction in chlorophyll a content was noted when the water stress levels increased gradually in the culture media. The results showed that chlorophyll a content of seedling was affected by the different water stress levels. Effect of drought stress on reduction of chlorophyll content of other plants *in vitro* has previously been reported by Molassiotis *et al*. (2006). There was a remarkable relationship between PEG concentration and chlorophyll a content.

The maximum chlorophyll b content was 0.48 mg/g at control treatment followed by 0.46 mg/g at 30 g/l PEG treatment (Figure 8). Minimum chlorophyll b content was 0.16 mg/g at 60 g/L PEG treatment among the 0-60 g/L treatments. Reduction of chlorophyll b content was observed when the PEG concentration increased gradually in the culture media. Remarkable reduction of chlorophyll content is reported in drought-stressed tomato plants (Hayat *et al*., 2008). Similar result was also reported by Meher *et al.* (2018) in chlorophyll content of peanut leaves. Regression model exhibited that there was a negative significant association between two variables of the measured chlorophyll b content of tomato seedling with different concentration of PEG solutions supplemented to the culture media. Liner slope value of the model was -0.005. Percentage of the chlorophyll $_b$ variation with the model determined as R-Squared value of 0.807. Chlorophyll a content was more than the chlorophyll b content of the seedling grown under stressed conditions (Figure 8). Hsu and Kao (2003) mentioned that Chl a is more sensitive than Chl b to PEG-induced water stress. Younis *et al*. (2000) stated that the reduction in chlorophyll at declining plant water potential may be due to sensitivity of the pigment to increasing environmental stress such as drought.

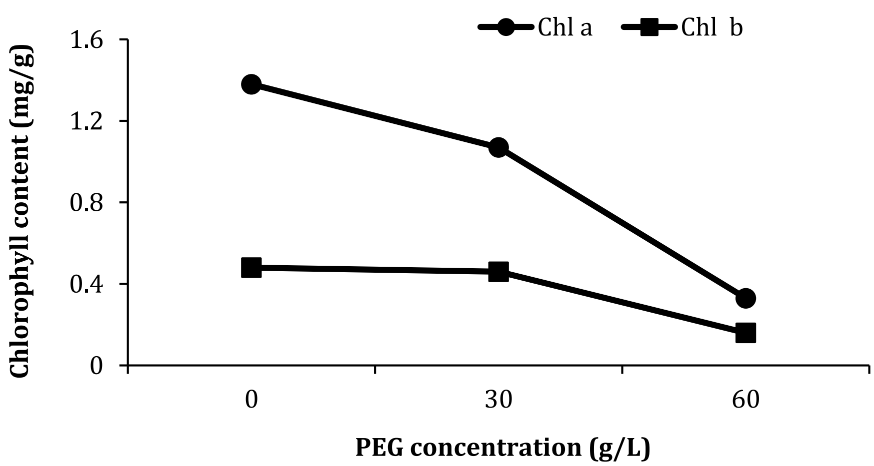

Figure 8: Chlorophyll a and b contents of leaves of tomato seedlings with different PEG concentrations after 4 weeks of culture

The maximum total chlorophyll content was found as 1.86 mg/g at control treatment (0 g/L) followed by 1.53 mg/g at 30 g/L PEG treatment. The minimum total chlorophyll content was found as 0.48 mg/g at 60 g/L PEG treatment, among the 0-60 g/L treatments. When the water stress levels increased gradually in the culture media results showed in the reduction of total chlorophyll content. Hsu and Kao (2003) reported that PEG induced water stress cause decrease in total chlorophyll content in rice leaves. The negative regression coefficient between the PEG concentration and total chlorophyll content of leaves (R^2 = -0.023) revealed that the increasing of PEG concentration has resulted in a decrease in the mean value of the total chlorophyll content. Results were in agreement with Mafakheri *et al.* (2010) who also reported that water stress reduces chlorophyll levels and photosynthetic activity.

Correlation between estimated parameters

Pearson correlation coefficients of the morphological parameters measured per *in vitro* seedling of combined data on water stressed and control conditions are presented in Table 1.

Table 1: Correlation between measured characteristics of the *in vitro* grown tomato seedlings

Parameter	SL	RL	FW	DW	CHL a	CHL b	TCHL
SL	1.000	-	-	-	-	-	-
RL	0.883**	1.000	-	-	-	-	-
FW	0.973**	0.941**	1.000	-	-	-	-
DW	0.910**	0.986**	0.964**	1.000	-	-	-
CHL a	0.904**	0.726**	0.840**	0.751**	1.000	-	-
CHL b	0.816**	0.628*	0.729**	0.651*	0.976**	1.000	-
TCHL	0.886**	0.705*	0.816**	0.729**	0.999**	0.986**	1.000

* significant at p=0.01 and ** significant at p=0.001; SL = shoot length (cm), RL = root length (cm), FW = fresh weight (mg), DW = dry weight (mg) CHL a = chlorophyll a (mg/g), CHL b = chlorophyll b (mg/g), TCHL = total chlorophyll (mg/g).

There was a positive and strong significant correlation between shoot and root length according to the Pearson correlation ($p<0.001$) in which correlation coefficient was 0.883 for the combined data. The root: shoot ratio represents the partitioning of biomass between root and aerial parts and it is an important index for evaluating plant health. Well-developed roots of the seedling help in nutrient and water absorption (Stavang *et al*., 2010). A strong positive correlation with significant effect was noted between shoot length and fresh weight of seedling for the combined data according to the Pearson correlation. Model indicated the coefficient correlation r = 0.973. There was a moderate positive correlation with significant relationship between the fresh weight of seedling and the chlorophyll a content of the seedlings. Correlation coefficient value for the combined data was 0.751.

Conclusion

In the present study, *in vitro* response of tomato *cv*. KC-1 was observed for water stress by using MS media containing different concentrations of PEG supplemented with 2 mg/L BAP. The *in vitro* response of seed germination and seedling performance were better in the MS medium fortified with 2 mg/L BAP and 0 mg/L PEG treatment. The results revealed that germination and seedling growth under *in vitro* method were drastically reduced on 60 g/L and 90 g/L PEG concentrations. With the PEG concentrations in the media, seed germination, shoot length, root length, fresh weight, dry weight, and chlorophyll a, chlorophyll b and total chlorophyll contents were decreased gradually. The results provided evidence that PEG-induced water stress has negatively affected the measured characteristics of the *in vitro* grown tomato seedling.

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