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Morpho-physiological analysis in strawberry (*Fragaria* x *ananassa* L.) under PEG (Polyethylene glycol) induced drought stress

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Abstract
A study on morpho-physiological analysis in Strawberry (*Fragaria* x *ananassa* L.) under PEG (Polyethylene Glycol) induced drought stress was carried out in a low cost net house of College of Horticulture and Forestry, Central Agricultural University, Pasighat, East Siang District, Arunachal Pradesh, India. The experiment consisted of two factors, the varieties (Camarosa, Festival and Winterdawn) and the PEG (0%, 20% and 40%) respectively and the experiment was laid out in Factorial Completely Randomized Design (FCRD). Different growth parameters such as shoot and root length, fresh and dry weight of the shoot and root, Relative water content (RWC) and chlorophyll content of the leaves were analyzed. Results showed that shoot and root length, fresh and dry weight of the shoots and the roots and RWC % decreases as a result of drought stress as induced by different levels of PEG. Among the varieties, Camarosa showed best performance.

Keywords: PEG, varieties, growth parameters, RWC, chlorophyll

Introduction

Strawberry (*Fragaria* x *ananassa* L.) is a fruit crops of prime importance all over the world and has a basic chromosome number *x*=7. It belongs to Rosaceae family and is a fruit crop of great popularity worldwide (Ruiz *et al*., 2011) [1]. The United States is the major producer of strawberry in the world followed by Turkey, Spain, Egypt and Mexico (Benjamin Elisha Sawe, 2017) [2]. In India, it is mostly grown in Satara district, Mahabaleshwar, Wai and Panchagani areas of Maharashtra and some parts of Himachal Pradesh and Jammu and Kashmir and Meghalaya. The area under its cultivation is increasing due to its diverse ecological state and cultivars having tolerance to different environmental conditions.

Due to its considerable nutrient value, it has drawn most growers' attention to start strawberry cultivation in North-eastern region of India. But sadly, they are subjected to certain environmental stresses during its growth and development limiting its production. Among these, drought is one of the main environmental stresses that adversely affect plant growth, economic outcome, and environmentally sustainable productivity. Plant growth and development depends upon the prevailing environmental factors of the growing region, soil and water resource availability. Limited water resource mainly influenced plant water stress resulting into reduction in yield.

Plant growth and development depends upon the prevailing environmental factors of the growing region, soil and water resource availability. Limited water resource mainly influenced plant water stress resulting into reduction in yield. Water stress means lack of availability of enough water for plant's use leading to negative water potential in plants cells or tissue and the period of water stress is often known as drought. Water stress is frequently encountered by plants during certain periods of year. Drought stress are linked to different plant morphological and physiological traits like reduced plant size, early maturity, and also reduced leaf area (Rizza *et al*., 2004) [3]. Decreased in plant height can be considered as an important mechanism for preservation of the carbohydrates by plants for constant metabolism and accumulation of solute for osmotic adjustment (Sunkar and Bartels, 2005) [4]. Drought stress also results in reduced leaf area and shoot length but stimulates root length, leaf abscission, leaf wax deposition, the number of leaves per plant, leaf size, and leaf longevity (Shao *et al*., 2008; Jamali *et al*., 2011; Hussein *et al*., 2007) [5, 6, 21].

Earlier studies have been carried out to identify drought tolerance ability in different crop species (Bota *et al*., 2001) [7], but the changes in morphological, physiological and biochemical aspects of strawberry plants imposed with drought stress is yet to be studied (Klamkowski

and Treder, 2008) [8]. So with theses in view, the present study is conducted to evaluate the changes in morpho-physiological attributes of strawberry under drought stress.

Materials and Method

The experiment was carried out in a low cost net house of College of Horticulture and Forestry, Central Agricultural University, Pasighat, East Siang District, Arunachal Pradesh, India, situated between 28°04`N latitude and 95°22`E longitude having an elevation of 153 meters above the mean sea level.

One month old strawberry runners were collected from Zopar Pvt. Limited, Shillong, Meghalaya. The experiment was laid out in Factorial Completely Randomized Design (FCRD). The experiment consisted of two factors, the cultivars as the first factor with 3 different varieties (Camarosa, Festival and Winterdawn) and the PEG (Polyethylene glycol) as the second factor with 3 levels (0%, 20% and 40%) respectively. In order to create a homogenized medium, the runners are grown in river sand. First, the sand was washed thoroughly with water followed by soaking overnight in an alkali solution (1N NaOH) and once again washed with water to remove the alkali. The same process is repeated with an acid solution (1N HCl) and washed repeatedly till all the organic matter has been removed from the sand and the pH is balanced. The sand was then placed in a hot air oven till it is completely dried where it was then transferred to clean polybags and placed in a dry area in a shaded net house. The runners were transplanted to clean polybags filled with sand and cocopeat (2:1). Each polybag contained 3 plants of the same cultivar and 10 pots were considered for each treatment. The transplanted runners was irrigated with a nutrient solution as suggested by Hoagland and Arnon, 1950, twice a week till they have established themselves in the sand and cocopeat mixture and are ready for treatment application. When the plants have been established in the pots, they were irrigated with nutrient solution containing the respective concentrations of polyethylene glycol (PEG). The nutrient solution containing the treatments was given twice a week to each pot. Irrigation with distilled water was also done whenever necessary.

Strawberry plant samples were collected after 15 days of treatments and were analysed in the Biotechnology Lab, Department of Basic Sciences and Humanity, College of Horticulture and Forestry, Pasighat. Statistical analysis of the data was carried out by using OPSTAT (http://14.139.232.166/opstat/default.asp). The data obtained from different observations during the field experimentation and laboratory analysis were subjected to the analysis of variance by Complete Randomized Design with factorial concept. Significance and non-significance of the variance due to the different treatments were determined by calculating the respective 'F' values (Gomez and Gomez, 2010) [9].

Observations Recorded

Growth parameters

To determine the growth of the strawberry plantlets grown under drought stress, the plantlets were uprooted 15 days after the treatment. The roots and shoots were separated and the lengths of the roots and shoots as well as their weights were determined based on 10 random samplings in triplicate. Shoot and root length of ten random plants were measured from the highest tips till the end of the crown with the help of centimeter scale in cm and their fresh weight was measured with the help of weighing balance in grams (g) and their average was calculated. Shoots and roots of the uprooted plants were washed and kept in hot air oven for 48 hours at 80 °C and thereafter dry matter of per plant was recorded. Average was worked out and expressed as shoot and root dry matter gram per plant (g/plant).

Relative water content of shoot (%)

Relative water content (RWC) was measured in roots and shoots of control as well as stressed Strawberry plants by using the method of Weatherly (1950) with some modifications. Root as well as shoot samples were weighed (fresh wt.) and then placed in water saturated vial at 5 ºC for 48 h and weighed (turgid wt.). The samples were dried in an oven at 80 ºC for 48 h and dry weights (dry wt.) were obtained.

Relative water content was calculated using the formula:

RWC= (FW-DW) / (TW -DW) × 100

where, FW= Fresh weight,

DW = Dry weight and

TW = Turgid weight.

Results and Discussion

Growth Parameters

Shoot Length (cm)

Shoot length after 15 days of water stress treatment with PEG was found to be significantly influence. The different levels of drought stress treatments resulted in decrease shoot length at the end of the observation. The highest shoot length was observed in 0% PEG level followed by 20% PEG level and the lowest shoot length was observed in 40% PEG level respectively (Table 1). After 15 days of treatment, different varieties also showed significant differences in shoot length. The highest shoot length was observed in Camarosa variety followed by Winterdawn and the minimum shoot length was found in Festival respectively (Table 1).

The combine effect of drought stress treatments with PEG and varieties remarkably reduces shoot length of the treated plants. The highest shoot length was observed in Camarosa treated with 0% PEG levels (23.36 cm) which is followed by Camarosa treated with 40% (21.67 cm) and the lowest was recorded in Festival treated with 40% PEG level (14.16 cm). It was evident that the interaction between PEG and varieties leads to decrease shoot growth in Camarosa, Festival and Winterdawn (Table 1). More profound decrease was observed in Festival as compared to Camarosa and Winterdawn. A percent decrease of 4.02% and 7.23% was recorded in Camarosa, 8.17% and 12.97% in Festival, 1.58% and 3.96% in Winterdawn at 20% and 40% PEG levels respectively.

The results is in conformity with the works of Erdogan *et al.*, (2016) [20] on strawberries under water deficit stress who reported that diminishing water supply caused a gradual decrease in the plant growth. And also with the results of Wu *et al.*, (2008) [18] who reported that the plant height was reduced up to 25% in water stressed citrus seedlings.

Root Length (cm)

Root length was observed to be highly significant due to the treatments at the end of the observation i.e. 15 days after the treatment. Drought stress due to PEG treatments resulted in significant changes in the length of the root (Table 1). The root length was found to be maximal in plants treated with 0% PEG (10.56 cm) which is followed by 20% PEG (9.54 cm) and the minimal in plants treated with 40% PEG (8.63 cm) respectively. Higher PEG concentrations lead to more reduction in the length of the root. Different varieties were

found to alter the length of the root resulting in the decrease of root growth with maximum length in Camarosa (11.70 cm) followed by Winterdawn (10.37 cm) and the least in Festival (6.65 cm) respectively (Table 1).

The interaction between PEG treatments and varieties leads to sharp decrease in the root growth after 15 days of the treatments imposed. Root length was observed to be significantly different among the different treatments of PEG and varieties. It ranges from 5.91 cm to 12.64 cm. Maximum length of root was found in Camarosa treated with 0% PEG (12.64 cm) which is followed by Camarosa treated with 20% PEG (11.66 cm) respectively whereas the minimum length of root was observed in Festival treated with 40% PEG (5.91 cm). The variety Winterdawn showed a percent decrease of 6.35% and 19.15% in root length when exposed to drought stress while in Festival variety root length showed percent decrease of 17.53% and 23.25% as a result of 20% and 40% PEG treatments level showing the highest decrease percentage among the varieties. In case of Camarosa root length showed decreased percentage of 7.75% and 14.48% in 20% and 40% PEG level showing the least decrease percentage among the treatments.

The results of the present study is supported by the work of Hussein *et al.* (2016) who in their study on strawberry under drought and salinity reported that root length reduces due to drought. However, it is in a sharp contrast with the findings of Klamlowski and Treder (2006) [11] who reported that there is no differences in the length of root system between control and water stressed plants in strawberry (*Fragaria x ananassa* Duch. cv. 'Salut') under greenhouse conditions.

Table 1: Effect of Peg Levels and Varieties on Shoot and Root Length under Drought Stress Conditions

Shoot Length					Root Length				
Treatments	**Camarosa**	**Festival**	**Winterdawn**	**Mean(P)**	**Treatments**	**Camarosa**	**Festival**	**Winterdawn**	**Mean(P)**
P_0	23.36	16.27	17.68	19.1	P_0	12.64	7.7	11.33	10.56
P_{20}	22.42 (-4.02)	14.94 (-8.17)	17.4 (-1.58)	18.25	P_{20}	11.66 (-7.75)	6.35 (-17.53)	10.61 (-6.35)	9.54
P_{40}	21.67 (-7.23)	14.16 (-12.97)	16.98 (-3.96)	17.6	P_{40}	10.81 (-14.48)	5.91 (-23.25)	9.16 (-19.15)	8.63
Mean (V)	22.48	15.12	17.35		Mean (V)	11.70	6.65	10.37	
Factors	Factor P	Factor V	Factor (PXV)		Factors	Factor P	Factor V	Factor (Pxv)	
SE(d)	0.16	0.16	0.28		SE(d)	0.1	0.1	0.17	
CD (5%)	0.34	0.34	0.59		CD (5%)	0.21	0.21	0.36	
SE (m)	0.11	0.11	0.2		SE (m)	0.07	0.07	0.12	

[Figures in the parenthesis represent percent increase (positive) and decrease (negative)]

Shoot Fresh Weight (g)

Shoot fresh weight was significantly affected by water stress treatment with PEG and varieties. PEG treatments were found to decrease the fresh weight of shoot at its higher level and the treatments were highly significant between them. Shoot fresh weight was highest in 0% PEG treatment followed by 20% PEG and minimum in 40% PEG treatment respectively (Table 2). Shoot fresh weight was observed to be significantly different due to different varieties. Camarosa showed maximum fresh weight of shoot followed by Winterdawn and the minimum shoot fresh weight was recorded in Festival (Table 2) respectively. The interaction effect of PEG treatment and varieties showed significant differences in between the treatments. The highest value recorded for shoot fresh weight was in Camarosa with 0% PEG level followed by Camarosa with 20% PEG level and the lowest value observed for shoot fresh weight is in Festival treated with 40% PEG level which is at par with Camarosa treated with 40% PEG (Table 2).

Shoot fresh weight decreases in all the treatments as a result of the interaction effect of PEG treatments and varieties at the end of the observation, i.e. 15 days after the treatment. A percentage decrease of 17.02% and 24.20% was observed in Camarosa, 22.56% and 39.49% in Festival and 14.69% and 36.49% in Winterdawn in response to the treatment 20% and 40% PEG levels. Higher stress levels induced more devastating effect on shoot fresh weight as compare to control. Festival showed highest decrease as compare to both Winterdawn and Camarosa at 20% and 40% PEG stress levels.

This is in agreement with Harb *et al.* (2005) [12] who found out that the gradual increase in PEG rates was negatively correlated with shoot fresh weight in their study on banana treated with polyethylene glycol (0, 3, 6, 9, 12 and 15%) and gamma irradiation (0, 10, 20, 30, 40, 50 and 60 Gy), alone or in combination.

Shoot Dry Weight (g)

Dry weight of shoot was noted to be significantly different due to drought stress as a result of PEG treatments as well as varieties. Shoot dry weight was found to be decreased due to PEG treatments. The highest value was found in 0% PEG followed by 20% PEG and the lowest value was recorded for 40% PEG respectively (Table 2). Different varieties affects shoot dry weight significantly by decreasing the values recorded in them at the end of the observation i.e. 15 days after the treatment. Camarosa showed maximum shoot dry weight followed by Winterdawn and the minimum was found in Festival respectively (Table 2). Both the PEG treatments and varieties results in significant differences in shoot dry weight. Maximum shoot dry weight was observed in Camarosa treated with 0% PEG followed by Camarosa treated with 20% PEG and the minimum dry weight of shoot was recorded in Festival treated with 40% PEG and also in Camarosa treated with 40% PEG (Table 2).

As evident from the Table 4, the variety Camarosa showed a percent decrease of 11.24% and 34.83%, Festival showed a percent decrease of 19.61% and 49.02% and Winterdawn showed a percent decrease of 8.16% and 36.73% respectively in response to 20% and 40% PEG treatment levels. The percent decrease of shoot dry weight was found to be highest in Festival and lowest in Camarosa variety at 20% and 40% PEG level and the decrease was most notable at 40% PEG than the other treatments.

The present finding is in conformity with the earlier work of Ghaderi *et al.* (2015) who reported that drought stress decreased leaf area, leaf dry matter, shoot dry matter and total dry matter in strawberry cvs. Kurdistan and Queen Elisa.

Root Fresh Weight (g)

Different PEG levels have significant differences on root fresh weight at 15 days after the treatment. Highest value was recorded in PEG level 0% followed by PEG level 20% and the lowest was recorded as against the PEG level 40% (Table 3). Different varieties were found to have significant

influence on root fresh weight at the end of the observation. The Winterdawn variety was found to have the maximum root fresh weight as against the minimum root fresh weight of Camarosa variety (Table 3). PEG levels and varieties interacted significantly among the different treatments on root fresh weight at 15 days after the treatment i.e. at the time of uprooting. Maximum root fresh weight was recorded in the treatment combination of WInterdawn treated with 0% PEG followed by Camarosa treated with 0% PEG whereas minimum root fresh weight was observed in Camarosa treated with 40% PEG (Table 3).

Root fresh weight decline in all the varieties in all the treatments showing more notable decline at higher PEG concentrations. At 20% and 40% PEG levels, Festival variety showed the highest decrease of 23.89% and 56.64% from the control. Camarosa showed a decrease percentage of 10.18% and 47.29% at 20% and 40% PEG level against 0% PEG while Winterdawn showed the decrease percentage of 11.68% and 35.39% of root fresh weight at 20% and 40% PEG levels respectively.

The present results is in conformity with Nezhadahmadi *et al.* (2015) who compared the morphological and physiological responses of three strawberry varieties under water deficit conditions in controlled and natural environments and concluded that drought stress and the duration of stress had negative effects on different growth parameters.

Root Dry Weight (g)

PEG treatment, varieties and the interaction between PEG and varieties produce significant variation in root dry weight among the treatments at the time of uprooting. Different PEG levels had significant effect on root dry weight after the uprooting of the stressed plants at 15 days after treatments was given. Highest root dry weight was recorded from 0% PEG followed by 20% PEG treatments whereas lowest root dry weight was found in 40% PEG treatment (Table 3). Different variety showed significant variation on root dry weight after 15 days of treatments. Root dry weight was recorded maximum in Winterdawn followed by Camarosa whereas minimum were found in Festival variety (Table 3). PEG and varieties interaction showed significant difference among the treatments at the end of the observation i.e. 15 days after the treatments. Maximum root dry weight was found in Winterdawn treated with 0% PEG level followed by Winterdawn treated with 20% PEG level and the minimum root dry weight was recorded from Camarosa treated with 40% PEG (Table 3).

Root dry weight was observed to decrease in all the varieties at 20% and 40% PEG treatment as against 0% PEG treatment. In Camaorsa, a percent decrease of 3.45% and 27.59% was observed, in Winterdawn, 7.84% and 35.29% decrease was recorded at 20% and 40% PEG levels and in Festival 9.09% and 36.36% at 20% and 40% PEG level were recorded respectively. Festival showed the highest decrease in root dry weight as compare to Camarosa and Winterdawn.

In another experiment by Medeiros *et al.* (2012) they also observed that root dry matter was decreased in Barbados cherry genotype 13-CPA under drought stress.

Relative Water Content (RWC) (%)

Relative water content (RWC) is an important attributes for water status in plants that have direct correlation with plant's photosynthetic activities. Due to drought stress effect and varietal differences, RWC of the treated plant changes strikingly showing reduction of water content in the stressed plants. The relative water content of the leaves was found to be statistically different among the treatments due to PEG, Varieties and the interaction between PEG and varieties. Different levels of PEG treatments significantly alter the relative water content (RWC) of the stressed leaves at 15 days after the treatment. Relative water content was recorded to be maximum at 0% PEG followed by 20% PEG whereas minimum relative water content was observed in 40% PEG (Table 4). Different varieties were found to influence significant variations in Relative Water Content (RWC) of the leaves at the end the observations. Highest values were recorded in Winterdawn followed by Camarosa and the minimum was found in Festival variety (Table 4). PEG and varieties interacted significantly showing variations among the treatments at 15 days after the treatments. Relative Water Content (RWC) was found to be at maximum in Camarosa treated with 0% PEG which is at par with Winterdawn treated with 0% PEG whereas minimum Relative Water Content (RWC) was observed in Festival treated with 40% PEG which is at par with Camarosa treated with 40% PEG (Table 4).

The Relative Water Content (RWC) of the stressed leaves was found to decrease at 20% and 40% PEG levels in all the varieties. At 20% PEG level, Winterdawn showed least percent decrease followed by Camarosa and Festival i.e. 5.83%, 7.95% and 13.41% respectively while at 40% PEG, Camarosa showed least percent decrease followed by Winterdawn and Festival i.e. 11.39%, 23.97% and 27.15% respectively. Festival showed highest decrease of RWC at the end of the observation.

The results of the present investigation is in line with the works of Ghaderi *et al.* (2015) [13, 15] and Parvin *et al.* (2015) [15] on strawberry who reported that drought stress decreases relative water content. The results of Shokri *et al.* (2016) [16] who worked on two strawberry cultivars (Paros and Queen Elisa) under drought stress also reported reduced relative water content in both the varieties.

Table 2: Effect of peg levels and varieties on fresh and dry weight of shoot under drought stress conditions

Shoot Fresh Weight					Shoot Dry Weight				
Treatments	**Camarosa**	**Festival**	**Winterdawn**	**Mean(p)**	**Treatments**	**Camarosa**	**Festival**	**Winterdawn**	**Mean(p)**
P_0	3.76	1.95	2.11	2.61	P_0	0.89	0.51	0.49	0.63
P_{20}	3.12 (-17.02)	1.51 (-22.56)	1.8 (-14.69)	2.14	P_{20}	0.79 (-11.24)	0.41 (-19.61)	0.45 (-8.16)	0.55
P_{40}	2.85 (-24.20)	1.18 (-39.49)	1.34 (-36.49)	1.79	P_{40}	0.58 (-34.83)	0.26 (-49.02)	0.31 (-36.73)	0.39
MEAN (V)	3.24	1.55	1.75		MEAN (V)	0.76	0.39	0.42	
Factors	Factor P	Factor V	Factor (PXV)		Factors	Factor P	Factor V	Factor (PXV)	
SE(d)	0.03	0.03	0.05		SE(d)	0.007	0.007	0.013	
CD (5%)	0.06	0.06	0.11		CD (5%)	0.016	0.016	0.028	
SE (m)	0.02	0.02	0.04		SE (m)	0.005	0.005	0.009	

[Figures in the parenthesis represent percent increase (positive) and decrease (negative)]

Table 3: Effect of peg levels and varieties on fresh and dry weight of root under drought stress conditions

Root Fresh Weight					Root Dry Weight				
Treatments	**Camarosa**	**Festival**	**Winterdawn**	**Mean(P)**	**Treatments**	**Camarosa**	**Festival**	**Winterdawn**	**Mean(P)**
P_0	1.29	1.13	2.9	1.78	P_0	0.29	0.22	0.51	0.34
P_{20}	1.16 (-10.08)	0.86 (-23.89)	2.57 (-11.68)	1.53	P_{20}	0.28 (-3.45)	0.20 (-9.09)	0.47 (-7.84)	0.32
P_{40}	0.68 (-47.29)	0.49 (-56.64)	1.88 (-35.39)	1.02	P_{40}	0.21 (-27.59)	0.14 (-36.36)	0.33 (-35.29)	0.23
Mean (V)	1.04	0.83	2.45		Mean (V)	0.26	0.19	0.44	
Factors	Factor P	Factor v	Factor (PXV)		Factors	Factor P	Factor V	Factor (PXV)	
SE(d)	0.03	0.03	0.05		SE(d)	0.003	0.003	0.005	
CD (5%)	0.05	0.05	0.09		CD (5%)	0.007	0.007	0.011	
SE (m)	0.018	0.018	0.032		SE (m)	0.002	0.002	0.004	

[Figures in the parenthesis represent percent increase (positive) and decrease (negative)]

Table 4: Effect of peg levels and varieties on relative water content under drought stress conditions

Treatments	Camarosa	Festival	Winterdawn	Mean(p)
P_0	76.94	73.85	71.68	74.16
P_{20}	70.82 (-7.95)	63.95 (-13.41)	67.5 (-5.83)	67.42
P_{40}	68.17 (-11.39)	53.8 (-27.15)	54.5 (-23.97)	58.82
MEAN (V)	71.98	63.87	64.56	
Factors	Factor P	Factor V	Factor (PXV)	
SE(d)	0.92	0.92	1.59	
CD (5%)	1.93	1.93	3.35	
SE (m)	0.65	0.65	1.13	

[Figures in the parenthesis represent percent increase (positive) and decrease (negative)]

Conclusion

From the studies, we can conclude that drought stress as well as difference in varietal characters leads to alter different morpho-physiological attributes of strawberry plants. Different levels of PEG teatments and varieties cause certain changes in the growth attributes as well as in the relative water content (RWC) of strawberry. The treatments 0% and 20% PEG has fewer effects than the 40% PEG levels in almost all the parameters. Of the three varieties, Camarosa showed better performance in most of the morpho-physiological parameters as compared to Winterdawn and Festival variety.

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