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# THE IMPACT OF DROUGHT STRESS ON MORPHOLOGICAL AND PHYSIOLOGICAL PARAMETERS OF THREE STRAWBERRY VARIETIES IN DIFFERENT GROWING CONDITIONS

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This study was done to compare morphological and physiological responses of three strawberry varieties under water deficit conditions in controlled and natural environments. Plants of three strawberry varieties were grown in different soil moisture levels including 25% (severe stress), 50% (mild stress), and 75% (normal irrigation) and remained for 2 months as a duration of stress. It was observed that there were significant differences among varieties, drought stress regimes, environments, and duration of drought stress for different traits ($P<0.05$). Leaf area, total shoot length, leaf number, chlorophyll content, chlorophyll stability index, leaf expansion rate, and plant height were higher in natural condition compared to the protected environment under drought stress. These parameters were diminished significantly ($P\leq0.05$) under stress. Plants in natural condition produced thicker leaves than protected environment which indicated their higher resistance to drought condition. Severe stress (25%) reduced leaf thickness significantly compared toother drought treatments. Varieties cultivated in glasshouse condition produced higher leaf moisture under 25% of stress level. Leaf yield is one the most important traits which is related to fruit yield. Higher leaf yield leads to enhanced fruit yield. Plants showed higher performance in terms of leaf yield in natural environment compared to glasshouse. Leaf yield was higher under optimal soil moisture conditions, compared to 50% and 25% soil moisture levels in both environments. Fruit yield was higher in natural environment compared to glasshouse condition and plants showed lower fruit yield under stress conditions. Correlation analysis showed strong relationships among traits. In the two growing environments, leaf area and leaf thickness were significantly correlated (0.805**). A weak relationship was observed between chlorophyll stability index and chlorophyll content at significant level of 0.01 (0.297**). Relative leaf expansion rate had negative medium relationships with chlorophyll (-0.403**) and chlorophyll stability index (-0.291*). Significant relationships were seen between leaf yield and chlorophyll content (-0.537**), leaf yield and chlorophyll stability index (-0.374**), and leaf yield and relative leaf expansion rate (0.433**). Finally, fruit yield was significantly correlated with total shoot length (0.713**), leaf area (0.891**), plant height (0.661**), leaf thickness (0.561**), leaf number (0.509**), chlorophyll (0.440**), and leaf yield (0.801**). It is concluded that drought stress and the duration of stress had negative effects on different growth parameters. Moreover, plants cultivated in natural conditions were more capable of coping with stress compared to the plants grown in controlled environment.
**Keywords:** *Fragariaananassa*, moisture content, chlorophyll content, leaf physiology, growing conditions

## INTRODUCTION

Strawberry (*Fragariaananassa*L.) is one of the most important fruit crops all over the world. It belongs to Rosaceae family and is a fruit crop of great popularity worldwide (Amil-Ruiz *et al*., 2011). The spread of strawberry in the world for its considerable nutrient value has drawn most growers' attention. As of importance in the human diet, fresh fruits have received particular attention (Giovannoni, 2004). Sadly, various environmental elements limit the production of this crop. Drought limits plants normal growth and development which is a common characteristic of many regions and drought stress has become an important limiting factor for crop production and yield. Plant genotype, growth stage, severity and duration of the stress are important factors which play role in plants' responses to drought stress (Chaves *et al*., 2003). In order to withstand drought stress, strawberry exhibits morphological and physiological responses. There have been various studies on morphological and physiological changes in strawberry plants in response to water stress. Heschel and Rigions (2005) explained that plant height was highly dependent on the amount of photosynthesis and was very sensitive to environmental conditions. During screening of three strawberry cultivars against drought stress, Klamkowski and Treder (2008) observed that drought stress reduced the leaf area in all cultivars named Elsanta, Elkat and Salut. According to Rucker *et al*. (1995), drought can reduce leaf area which leads to diminished photosynthesis. Also, the number of leaves per plant, leaf size, and leaf longevity were reduced by water stress (Shao *et al*., 2008). More recently, Grant *et al*. (2010) showed substantial morphological variation of ten cultivars of strawberry under normal and water deficit conditions. They observed that responses to water deficits were not uniform across cultivars in which strawberry numbers and area of new leaves, dry mass of leaves and roots were significantly reduced under the water deficit treatment. Moreover, they claimed that small leaf area as a drought tolerant characteristic of strawberry cultivars can be of advantage for variety selection.Rizza *et al*. (2004) also expressed that there are a lot of characteristics linked to water stress like tiny plant size, early maturity, and diminished leaf area. Extension of leaves can be restricted under drought stress to get a balance among water status of crop tissue and the absorbed water by roots (Passioura, 1996). Drought is associated with reduction in leaf thickness (Santacruz and Cock, 1984). Leaves are generally thicker under water stress (Moreno-Sotomayor *et al*., 2002). Thick leaves are the characteristic features of drought stressed plants, as reported by Ristic*et al*. (1991) in Zea mays, Silva *et al*. (1999) in common bean, and Sam *et al*. (2000) in tomato. High leaf thickness is often related to drought tolerance (Chandra *et al*., 2004). Chlorophyll activity is critical in drought resistance which was declined during strong water stress in two varieties of strawberry (Ghaderi and Siosemardeh, 2011). In another study by Kirnak*et al*. (2001), chlorophyll content was reduced by 55% under drought stress conditions compared to control. The adverse effect of water stress on chlorophyll content has previously been shown for young peach trees by Steinberg *et al*. (1990). Relative leaf expansion rate is one of the most important traits for plant development during water stress treatment. Kirnak*et al*. (2001) observed that leaf expansion rates were decreased by water stress treatments in different eggplant varieties. Drought stress decreased leaf moisture content in different plant varieties (Kirnak*et al*., 2001). Water deficit occurs when there is water limitation in the root area which results in reduced leaf and fruit yields. Under field conditions, strawberry yield was diminished as an outcome of soil moisture deficit (Li *et al*., 2010). Klamkowski and Treder (2008) observed variations among three strawberry varieties in root development and yield under drought stress. In this study, it was tried to estimate the impact of different drought stress regimes and duration of stress on morphological and physiological responses of various strawberry varieties. Furthermore, different comparisons were made between natural and protected conditions to evaluate different responses of plants to drought stress.

## MATERIALS AND METHODS

***Plant materials*:** One month old seedling of three strawberry varieties namely Bogota, Chandler, and Festival were acquired from strawberry farm in Cameron Highlands, Pahang, Malaysia.

***Methods*:** The experiment was conducted in natural (Cameron Highlands, Pahang, Malaysia) and protected (Glasshouse, Institute of Biological Sciences, University of Malaya, Malaysia) environments following completely randomized design (CRD). Strawberry plants were grown in pails with suitable soil mixture and each pail was filled with 3 kilograms of soil. Plants were carefully cultivated in the center of the pail to let plants have a suitable space to grow. Three different regimes of soil moisture levels were applied namely 25% (severe stress), 50% (mild stress), and 75% (normal irrigation) with three replication. Each regime of soil moisture was fixed after measurements using soil moisture meter and irrigation was setup using this device. As far as stress intensity increases with time, 2 months duration of stress was applied to observe plants responses to the stress. Data was collected every 10 days in each of stressed and control plants. Fertilizer was applied in the ratio of 5-5-5 (N-P-K) or 7-7-7 (N-P-K) in the form of liquid. Fungicide (Thiram) and insecticide (Malathione) were sprayed on plants every two weeks alternatively to avoid existence of fungus and pests. parameters such as, Several morpho-physiogical traits such as plant height (PH), total shoot length (TSL), leaf area (LA), leaf thickness (LT), leaf number (LN), chlorophyll content (Chl), chlorophyll stability index (CSI), leaf moisture content (LMC), relative leaf expansion rate (RLER), and leaf yield (LY) were analyzed.

***Plant height (PH)*:** Measured from the soil base up to the tip of the plant.

***Total Shoot length (TSL)*:** Individual shoot length was measured.

***Leaf area (LA)*:** Calculated using the following equation (Birch *et al*., 1998; Montgomery, 1911).

Leaf Area ($cm^2$)

= leaf length × leaf width × 0.75 (the factor).

***Leaf thickness (LT)*:** Leaf thickness was measured by using a digital Vernier Caliper (150mm Digital VernierCaliper Gauge Micrometer).

***Leaf number*:** The numbers of leaves were counted.

***Chlorophyll content (Chl)*:** This parameter was measured by SPAD 502 Plus Chlorophyll Meter.

***Chlorophyll stability index (CSI)*:** This parameter was calculated following formula of Sivasubramaniawn (1992).

Chlorophyll stability index (%)

= CSI (%) = **Error! Reference source not found.**

***Leaf moisture content (LMC)*:** This parameter is used to estimate crops water status (Saura-Mas and Lloret, 2007).

Leaf moisture content (%): **Error! Reference source not found.**

Where: FW indicates leaves fresh weight and DW refers to dry weight (Saura-Mas and Lloret, 2007).

***Relative leaf expansion rate (RLER)*:** This trait was estimated following the formula of Kirnak*et al*. (2001) in which RLER = **Error! Reference source not found.** where

LA1, LA2 are the initial and final leaf areas and T1, T2 are the times of the two measurements.

***Leaf yield (LY)*:** Fresh leaves were weighed by a digital weighing balance (FX300i).

***Fruit yield (FY)*:** Fruits per plant were weighed.

***Statistical analysis*:** SPSS Version 21 and Excel 2013 software were used to analyze the data in this study.

## RESULTS

Morphological and physiological performances of strawberry plants were limited by water stress in different stress levels and duration of time. Significant differences were observed among varieties, treatments, duration of drought stress, and environments in terms of different traits (P<0.05) (Tables 1, 2, 3, 4).

According to these tables, there were significant differences among varieties in various traits. Different drought stress regimes were also significantly different for various parameters except for leaf thickness, leaf number, and leaf moisture content. There were significant differences between different environments at 0.05 level. Mean differences for different morphological and physiological traits in various treatment conditions and environments are shown in Tables 5, 6 and 7.

There were significant differences among varieties in terms of various parameters among drought stress treatments and environments (P<0.05). Different values for leaf area in three strawberry varieties in two different environments are demonstrated (Fig. 1a). According to this figure, it was observed that all of the genotypes produced higher leaf area in natural environment compared to glasshouse condition in which Bogota and Chandler produced the highest and lowest leaf area in both conditions, respectively.

**Table 1. Observation of various traits under different drought conditions and time duration**

| S.O.V | Leaf Area | | | | Total Shoot Length | | | | Plant Height | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Mean** | **SD** | **F-value** | **CV (%)** | **Mean** | **SD** | **F-value** | **CV (%)** | **Mean** | **SD** | **F-value** | **CV (%)** |
| Variety | 279.51 | 30.26 | 54.23* | 10.82 | 715.09 | 66.77 | 33.93* | 9.34 | 95.55 | 2.48 | 7.99* | 2.59 |
| Drought | 279.51 | 30.26 | 52.19* | 10.82 | 715.09 | 66.77 | 112.81* | 9.34 | 95.55 | 2.48 | 22.84* | 2.59 |
| Duration | 139.76 | 15.13 | 1.30ns | 10.82 | 357.54 | 33.38 | 1.229ns | 9.34 | 47.78 | 1.24 | 11.64* | 2.59 |
| Location | 419.27 | 45.39 | 2030.42* | 10.82 | 1072.63 | 100.16 | 1431.82* | 9.34 | 143.33 | 3.73 | 192.73* | 2.59 |

Level of significance: *=0.05, **= 0.01, NS= Not Significant

**Table 2. Observation of various traits under different drought conditions and time duration**

| S.O.V | Leaf Thickness | | | | Leaf Number | | | |
|---|---|---|---|---|---|---|---|---|
| | **Mean** | **SD** | **F-value** | **CV (%)** | **Mean** | **SD** | **F-value** | **CV (%)** |
| Variety | 3.65 | 0.141 | 72.25* | 3.88 | 134.12 | 10.91 | 19.62* | 8.13 |
| Drought | 3.65 | 0.141 | 0.699ns | 3.88 | 134.12 | 10.91 | 5.81* | 8.13 |
| Duration | 1.82 | 0.070 | 4.460* | 3.88 | 67.06 | 5.45 | 7.38* | 8.13 |
| Location | 5.47 | 0.212 | 545.96* | 3.88 | 201.18 | 16.36 | 332.13* | 8.13 |

Level of significance: *=0.05, **= 0.01, NS= Not Significant

**Table 3. Observation of different traits under various drought conditions and time duration**

| S.O.V | Chlorophyll Content | | | | Chlorophyll Stability Index | | | | Leaf Moisture Content | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Mean** | **SD** | **F-value** | **CV (%)** | **Mean** | **SD** | **F-value** | **CV (%)** | **Mean** | **SD** | **F-value** | **CV (%)** |
| Variety | 38.80 | 4.63 | 3.402* | 11.93 | 96.62 | 7.70 | 9.059* | 7.96 | 304.45 | 74.18 | 0.757ns | 24.36 |
| Drought | 38.80 | 4.63 | 14.163* | 11.93 | 96.62 | 7.70 | 5.067* | 7.96 | 304.45 | 74.18 | 0.127ns | 24.36 |
| Duration | 38.80 | 4.63 | 31.611* | 11.93 | 96.62 | 7.70 | 5.067* | 7.96 | - | - | - | - |
| Location | 38.80 | 4.63 | 84.828* | 11.93 | 96.62 | 7.70 | 5.067* | 7.96 | 304.45 | 74.18 | 0.777ns | 24.36 |

Level of significance: *=0.05, **= 0.01, NS= Not Significant

**Table 4. Observation of different traits under various drought conditions and time duration**

| S.O.V | Relative Leaf Expansion Rate | | | | Leaf Yield | | | | Fruit Yield | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Mean** | **SD** | **F-value** | **CV (%)** | **Mean** | **SD** | **F-value** | **CV (%)** | **Mean** | **SD** | **F-value** | **CV (%)** |
| Variety | -0.0053 | 0.0017 | 0.493ns | -32.07 | 12.58 | 4.11 | 1.461ns | 32.67 | 43.11 | 13.53 | 0.256ns | 31.38 |
| Drought | -0.0053 | 0.0017 | 17.34* | -32.07 | 12.58 | 4.11 | 9.996* | 32.67 | 43.11 | 13.53 | 3.046ns | 31.38 |
| Location | -0.0053 | 0.0017 | 14.68* | -32.07 | 12.58 | 4.11 | 380.72* | 32.67 | 43.11 | 13.53 | 86.45* | 31.38 |

Level of significance: *=0.05, **= 0.01, NS= Not Significant

**Table 5. Mean differences of different varieties, duration, and treatments for various traits under drought stress**

| Variety | Drought | Leaf Area – Glasshouse | Leaf Area – Natural | Total Shoot Length – Glasshouse | Total Shoot Length – Natural | Plant Height – Glasshouse | Plant Height – Natural |
|---|---|---|---|---|---|---|---|
| Bogota | 25% | 13.45d | 25.81b | 41.57c | 141.49cd | 14.42ab | 17.40ab |
| | 50% | 16.78c | 31.03a | 39.54c | 153.05c | 14.69ab | 18.15ab |
| | Control | 25.09b | 31.58a | 49.24bc | 222.97b | 13.47b | 18.49ab |
| Chandler | 25% | 10.36e | 15.62d | 50.16bc | 139.20cd | 13.60b | 14.49bc |
| | 50% | 17.92c | 19.06cd | 63.99ab | 143.38cd | 16.29a | 15.64b |
| | Control | 29.33a | 21.50c | 77.38a | 189.73bc | 15.88a | 16.10b |
| Festival | 25% | 10.59e | 24.75bc | 44.68c | 143.74cd | 12.42b | 17.11ab |
| | 50% | 13.53d | 26.05b | 54.82b | 205.62c | 14.18ab | 18.88ab |
| | Control | 23.96bc | 30.51ab | 72.24a | 312.46a | 14.92ab | 20.53a |

Note: different letters indicates significant difference, same letters indicates no significant difference

**Table 6. Mean differences of different varieties, duration and treatments for various traits under drought stress**

| Variety | Drought | Leaf Thickness – Glasshouse | Leaf Thickness – Natural | Leaf Number – Glasshouse | Leaf Number – Natural | Chlorophyll Content – Glasshouse | Chlorophyll Content – Natural | Chlorophyll S.I. – Glasshouse | Chlorophyll S.I. – Natural |
|---|---|---|---|---|---|---|---|---|---|
| Bogota | 25% | 0.56a | 0.83a | 12.25c | 43.02a | 35.17ab | 41.84a | 96.94ab | 100.70a |
| | 50% | 0.50ab | 0.85a | 11.07cd | 36.26ab | 36.99ab | 40.23ab | 102.29a | 96.80a |
| | Control | 0.49ab | 0.85a | 14.48b | 37.66ab | 36.43ab | 41.56a | 100.00a | 100.00a |
| Chandler | 25% | 0.45b | 0.58bc | 15.21b | 20.58d | 34.75ab | 34.75b | 87.92b | 91.19ab |
| | 50% | 0.49ab | 0.62b | 16.25ab | 23.81cd | 39.03a | 40.33ab | 99.00a | 92.72ab |
| | Control | 0.50ab | 0.61b | 19.41a | 24.97c | 39.33a | 43.60a | 100.00a | 100.00a |
| Festival | 25% | 0.47ab | 0.72ab | 13.70bc | 24.38c | 35.82ab | 39.20ab | 91.37ab | 94.75ab |
| | 50% | 0.50ab | 0.68ab | 15.16b | 25.13c | 36.90ab | 37.48b | 94.31ab | 91.26ab |
| | Control | 0.52a | 0.72ab | 18.76a | 30.26b | 39.01a | 41.07a | 100.00a | 100.00a |

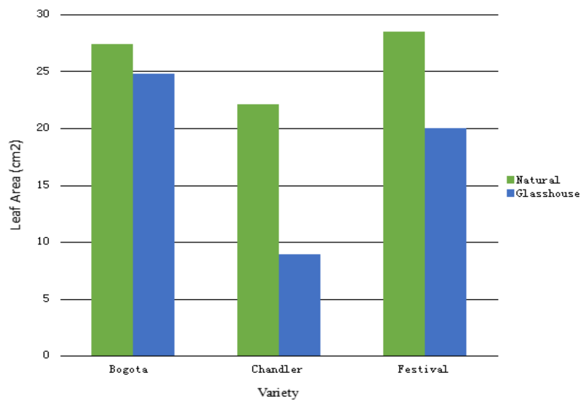
Note: different letters indicate significant differences, same letters indicate no significant differences

**Table 7. Mean differences of different varieties, duration, and treatments for various traits under drought stress**

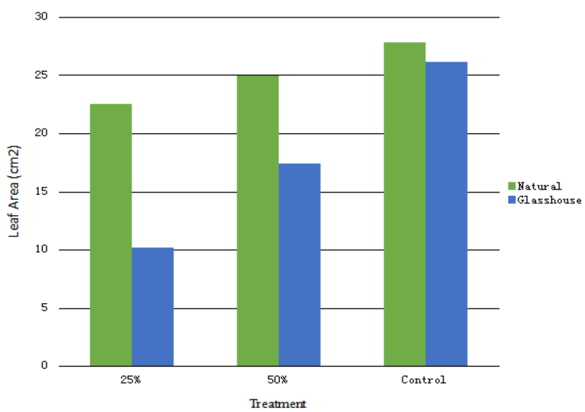
| Variety | Drought | Leaf Moisture Content – Glasshouse | Leaf Moisture Content – Natural | R. Leaf Expansion Rate – Glasshouse | R. Leaf Expansion Rate – Natural | Leaf Yield – Glasshouse | Leaf Yield – Natural | Fruit Yield – Glasshouse | Fruit Yield – Natural |
|---|---|---|---|---|---|---|---|---|---|
| Bogota | 25% | 217.63e | 349.90a | -0.0153ef | -0.0050f | 1.08c | 19.35cd | 35.41ab | 54.58bc |
| | 50% | 240.46de | 358.33a | -0.0273g | -0.0036ef | 1.45bc | 23.89bc | 34.45ab | 60.12b |
| | Control | 253.72d | 304.90ab | 0.0033b | 0.0056b | 1.63b | 34.90a | 35.50ab | 69.85a |
| Chandler | 25% | 400.00a | 255.60c | -0.0206f | -0.0086g | 0.746c | 20.03c | 34.67ab | 50.16c |
| | 50% | 290.27c | 290.72b | -0.0156ef | -0.0010d | 1.46bc | 21.39bc | 36.89ab | 55.71bc |
| | Control | 362.71ab | 247.40c | 0.0103a | 0.0116a | 1.70b | 30.99ab | 41.14a | 65.05ab |
| Festival | 25% | 380.55ab | 247.48c | -0.0143e | -0.0043f | 0.686cd | 20.30c | 33.11b | 45.09d |
| | 50% | 350.46ab | 252.29c | -0.0093d | -0.0026e | 1.68b | 17.44d | 36.69ab | 63.27ab |
| | Control | 326.33b | 351.46a | -0.0033c | 0.0036c | 2.20a | 25.65b | 40.09a | 70.16a |

Note: different letters indicates significant difference, same letters indicates no significant difference

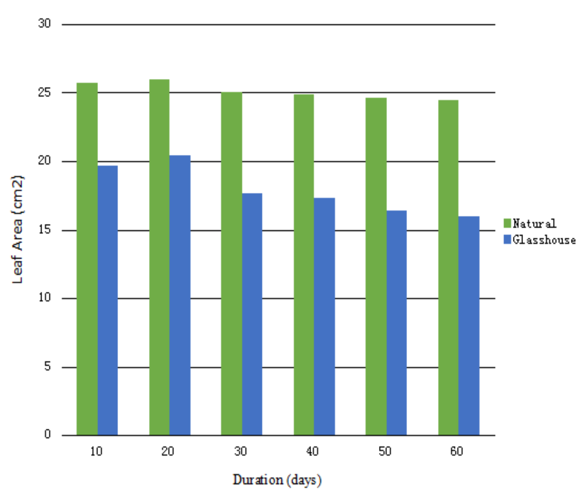
Plant varieties cultivated in natural condition performed better leaf area compared to protected environment in all soil moisture levels (Fig. 1b). The lowest results were observed when varieties treated with 25% of soil moisture in both environments. Varieties had the highest leaf area under normal irrigation in both glasshouse and natural conditions. In terms of duration of stress, in both environments, plant varieties were exposed to 60 days of drought stress and the result indicated that with each increase in time duration, leaf area was decreased (Fig. 1c). Varieties gave the best leaf area results in natural condition in all time duration compared to protected environment. The highest leaf area was observed in 10 days after drought treatment and 60 days duration showed the lowermost leaf area value in all varieties in both environments (Fig. 1c). Drought stress affected strawberry plants total shoot length which is one of the most important parameters. Bogota and Festival showed the lowest and highest shoot length in two environments under drought stress (Fig. 2a). Plants produced the lowest shoot length in 25% of treatment in comparison with other treatments and those plants which were cultivated in normal condition performed the best rates (Fig. 2b). In terms of time duration, in both cultivation environments, all three varieties showed the lowermost and highest rates of shoot lengths when treated with 60 days and 10 days duration (Fig. 2c). All three varieties had higher shoot length in 10 days after drought stress and the rate was decreased by the passage of time in all varieties.




**1a**

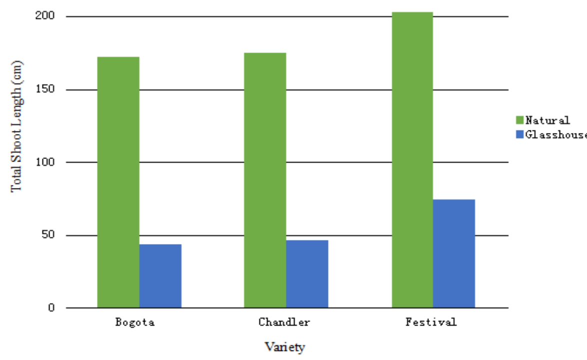



**1b**

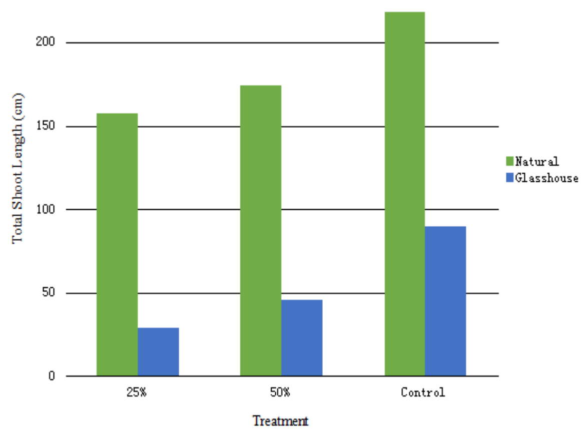



**1c**

**Figure 1. The effect of drought stress on plants leaf area**




**2a**

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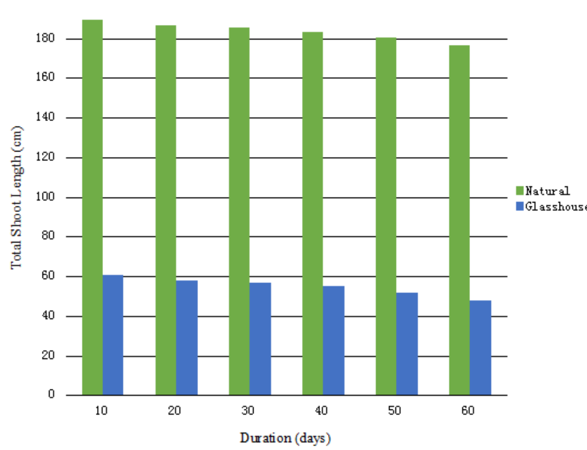
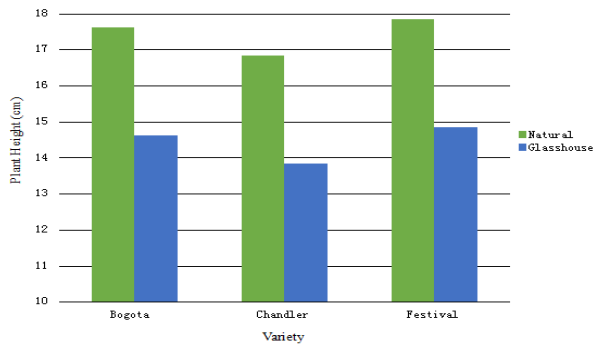


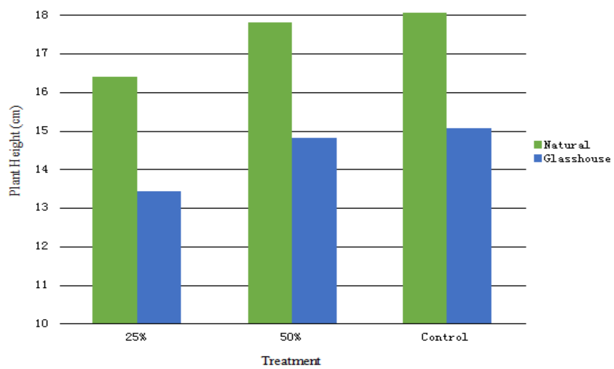

**2c**

**Figure 2. The effect of drought stress on plants shoot length**

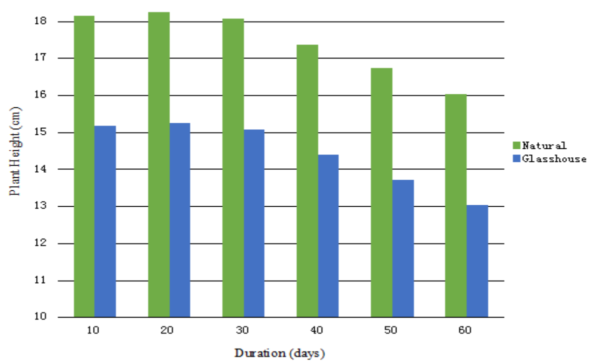
In terms of plant height, Chandler and Festival showed the lowest and highest plant height in two environments under drought stress (Fig. 3a). Plants produced the lowest plant height in 25% of treatment in comparison with other treatments and those plants which were cultivated in normal condition performed the best rates (Fig. 3b). In terms of time duration, in both cultivation environments, there were decreasing trends in terms of plant height from 10 to 60 days duration in both natural and glasshouse conditions (Fig. 3c).




**3a**



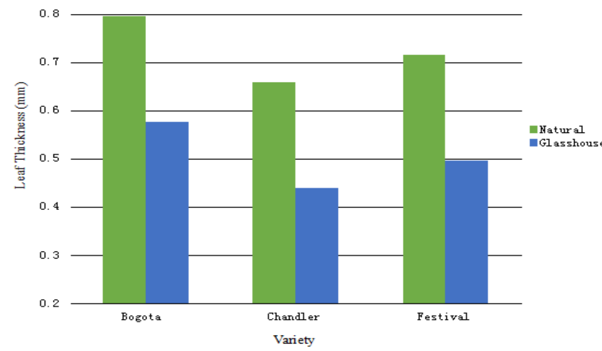

**3b**



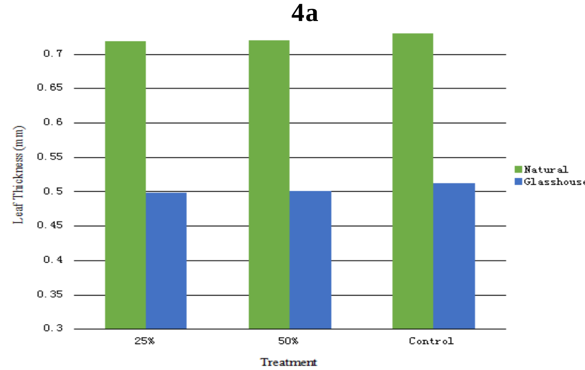

**3c**

**Figure 3. The effect of drought stress on plants height**

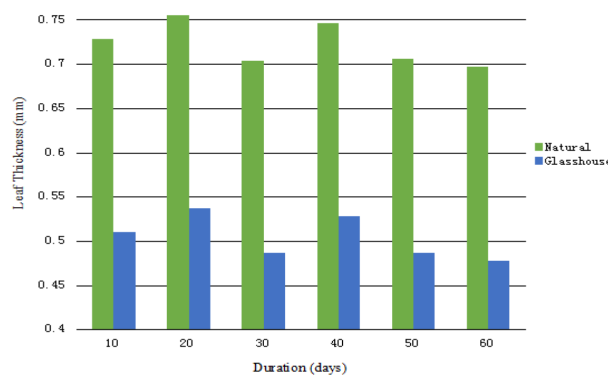
All three varieties showed the highest and lowermost rates of plant height when treated with 10 days and 60 days duration (Fig. 3c). All three varieties had higher plant height in 10 days after drought stress and the rate was decreased by the passage of time in all varieties. In terms of leaf thickness, all varieties presented lower results in protected environment compared to natural conditions (Fig. 4a).




**4a**



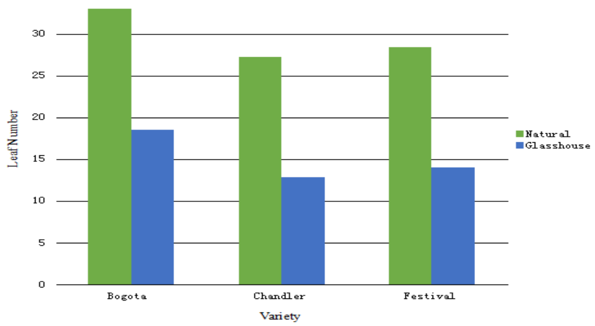

**4b**



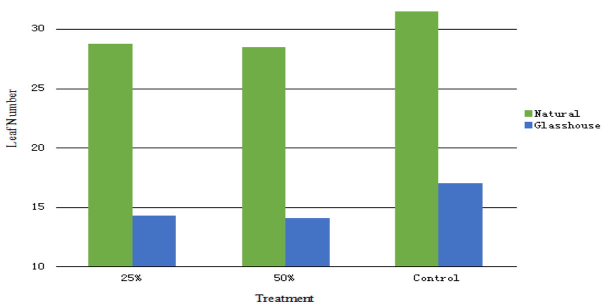

**4c**

**Figure 4. The effect of drought stress on plants leaf thickness**

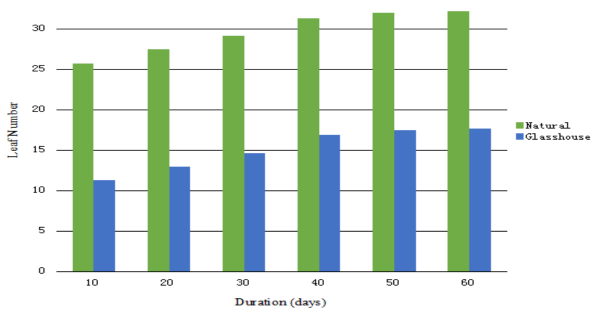
Chandler and Bogota had the lowermost and highest leaf thickness rates respectively in both conditions. Plants produced the lowest leaf thickness in 25% of treatment in comparison with other treatments and those plants which were cultivated in normal condition performed the best rates (Fig. 4b). In terms of time duration, there were fluctuations in leaf thickness from 10 to 60 days duration in both environments. However, after 40 days of treatment, plants started demonstrating their thinner leaves in glasshouse and natural conditions (Fig. 4c). In terms of leaf number, Chandler and Bogota showed the lowest and highest leaf number in two environments under drought stress (Fig. 5a).




5a




**5b**

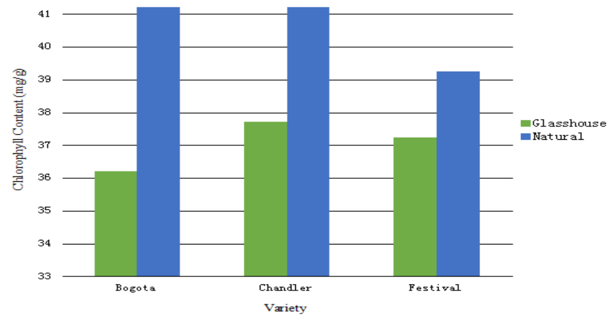



**5c**

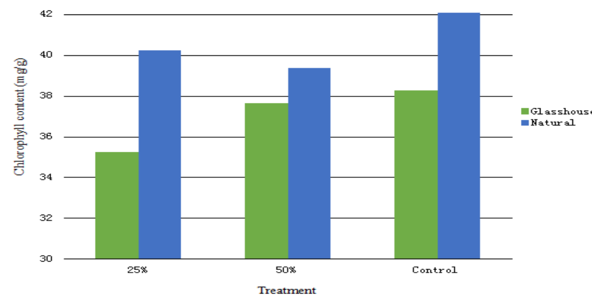
**Figure 5. The effect of drought stress on plants leaf number**

Plants produced the lower leaf number in 25% and 50% of treatments in comparison with control and those plants which were cultivated in normal condition performed the best rates (Fig. 5b). In terms of time duration, in both cultivation environments, there were increasing trends in terms of leaf number from 10 to 60 days duration in both natural and glasshouse conditions (Fig. 5c). When strawberry varieties were remained for 60 days under drought stress, leaf number was increased after 60 days of drought condition. Surprisingly, leaf number was lower in 10 days after drought stress, followed by 20, 30, 40, 50, and 60 days in all varieties and environments (Fig. 5c).

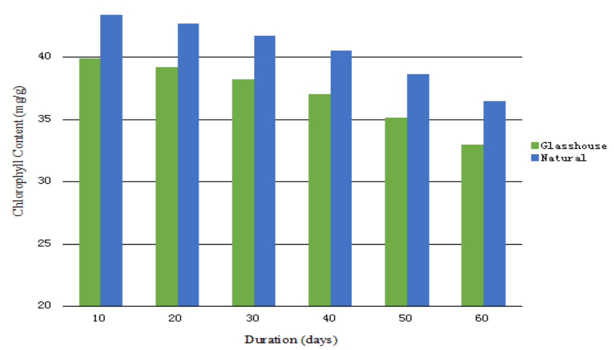
Different values for chlorophyll content in three strawberry varieties in two different environments are demonstrated (Fig. 6a). According to this figure, it was observed that all of the genotypes produced higher chlorophyll content in natural environment compared to glasshouse condition in which Chandler produced the highest chlorophyll content in both conditions. Plant varieties cultivated in natural condition performed better chlorophyll content results compared to protected environment in all soil moisture levels (Fig. 6b).




**6a**



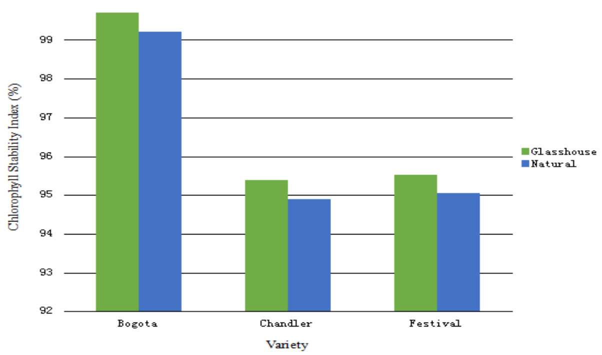

**6b**



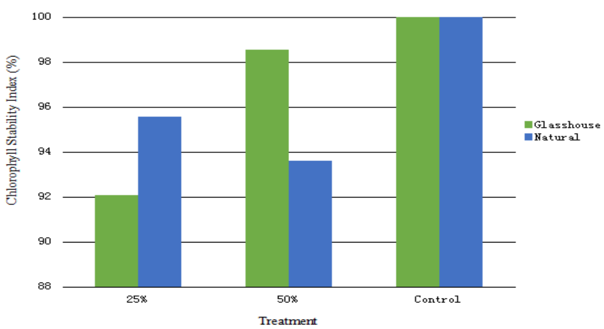

**6c**

**Figure 6. The effect of drought stress on plants chlorophyll content**

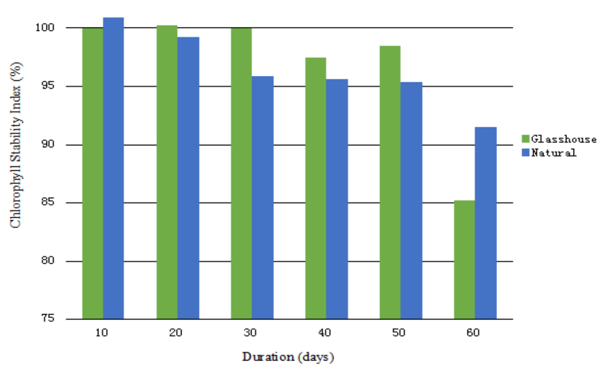
The lowest results were observed when varieties treated with 25% and 50% of soil moisture in glasshouse and natural environments respectively. Varieties had the highest chlorophyll content under normal irrigation in both glasshouse and natural conditions. In terms of duration of stress, in both environments, plant varieties were exposed to 60 days of drought stress and the result indicated that with each increase in time duration, chlorophyll content was decreased (Fig. 6c). Varieties gave the best chlorophyll content results in natural condition in all time duration compared to protected environment. The highest chlorophyll content was observed in 10 days after drought treatment and 60 days duration showed the lowermost chlorophyll content in all varieties in both environments (Fig. 6c). Drought stress affected strawberry plants chlorophyll stability which is one of the most important parameters. In the current investigation, Bogota and Chandler showed the highest and lowest chlorophyll stability index in two environments under drought stress (Fig. 7a). Plants produced the lowest and highest chlorophyll stability index in 25% and 50% of treatments in glasshouse and natural conditions respectively. Plants cultivated in normal condition performed the best rates (Fig. 7b).




**7a**




**7b**

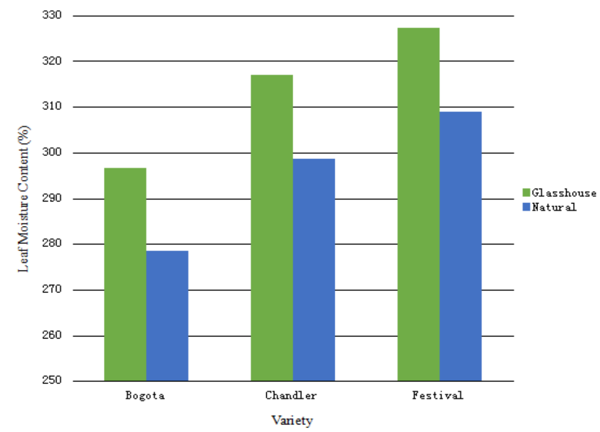



**7c**

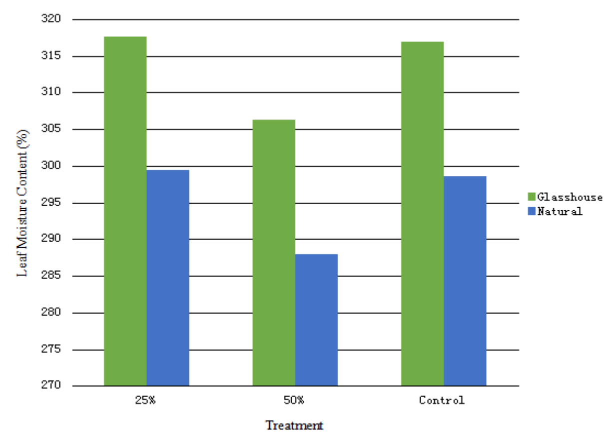
**Figure 7. The effect of drought stress on plants chlorophyll stability index**

In terms of time duration, in both cultivation environments, all three varieties showed the lowermost and highest rates of chlorophyll stability index when treated with 60 days and 10 days duration (Fig.7c). All three varieties had higher chlorophyll stability index in 10 days after drought stress and the rate was decreased by the passage of time in all varieties.

In terms of leaf moisture content, varieties cultivated in glasshouse condition produced higher leaf moisture in which Bogota and Festival showed the lowest and highest plant height in two environments under drought stress (Fig. 8a).



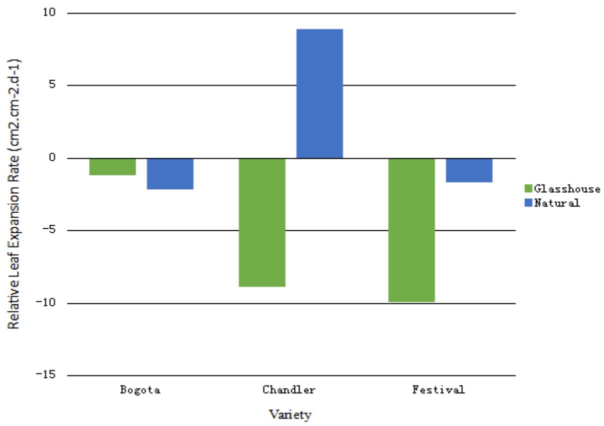

**8a**



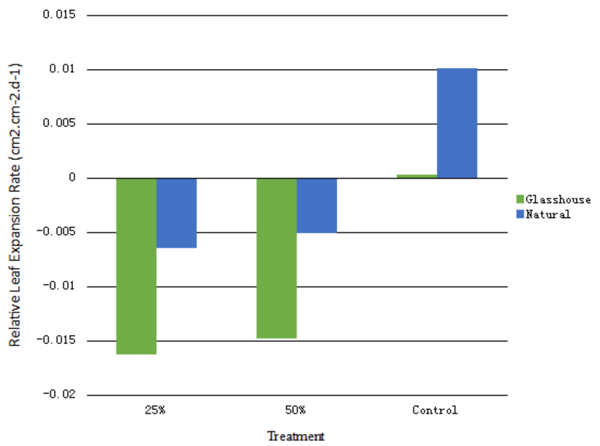

**8b**

**Figure 8. The effect of drought stress on plants leaf moisture content**

Plants produced the lowest leaf moisture content in 50% of treatment in comparison with other treatments and those plants which were cultivated in 25% condition performed the best rates (Fig. 8b). Varieties had better leaf expansion rates in natural condition compared to glasshouse. Chandler demonstrated better leaf expansion compared to other varieties while Bogota had higher expanded leaves in glasshouse condition which was negative (Fig. 9a). Plants were observed to have negative leaf expansion rate when treated with 25% and 50% drought treatments in both locations (Fig. 9b).



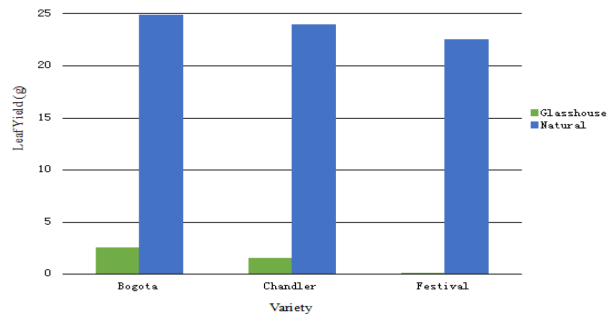

**9a**



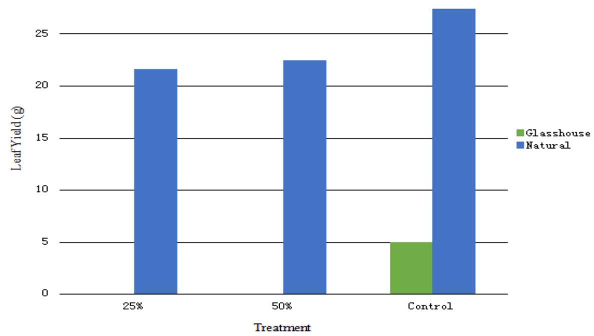

**9b**

**Figure 9. The effect of drought stress on plants relative leaf expansion rate**

Varieties cultivated in normal condition showed higher and positive expansion rate. All varieties had lower expansion rate in 25% drought treatment compared to 50% and control in both environments. Plants showed higher performance in terms of leaf yield in natural environment compared to glasshouse. Bogota performed the best leaf yield while Festival demonstrated the lowest rate in both locations (Fig. 10a). Leaf yield was higher in normal irrigation compared to 50% and 25% soil moisture levels respectively in both locations (Fig. 10b). Plants had the lowest yield in 25% treatment compared to 50% and control treatments. Fruit yield was higher in natural environment compared to glasshouse condition and plants showed lower fruit yield under stress conditions (Figs. 11a and11b).

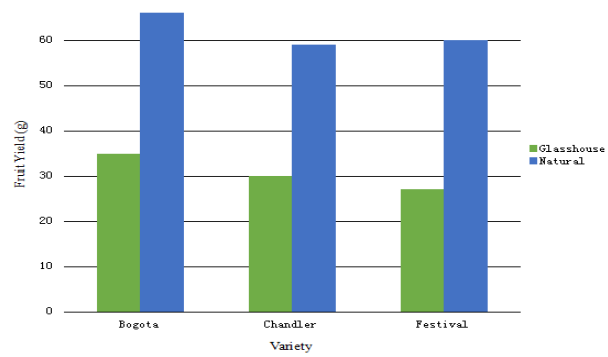



**10a**

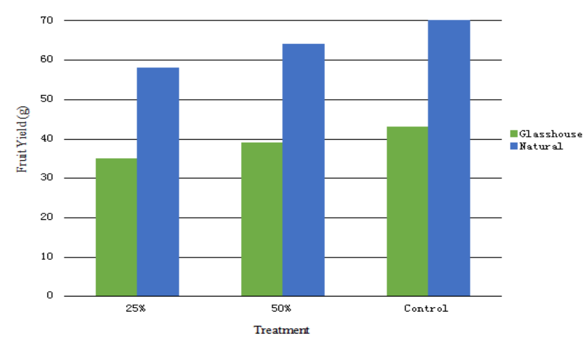



**10b**

**Figure 10. The effect of drought stress on plants leaf yield**




**11a**




**11b**

**Figure 11. The effect of drought stress on plants fruit yield**

**Table 8. Correlation analysis of different parameters with each other**

| Parameters | TSL | LA | PH | LT | LN | Chl | CSI | LMC | RLER | LY | FY |
|---|---|---|---|---|---|---|---|---|---|---|---|
| TSL | 1.000 | | | | | | | | | | |
| LA | 0.868** | 1.000 | | | | | | | | | |
| PH | 0.717** | 0.721** | 1.000 | | | | | | | | |
| LT | 0.632** | 0.805** | 0.559** | 1.000 | | | | | | | |
| LN | 0.626** | 0.734** | 0.521** | 0.653** | 1.000 | | | | | | |
| Chl | 0.404** | 0.419** | 0.503** | 0.330** | 0.309** | 1.000 | | | | | |
| CSI | 0.112ns | 0.053ns | 0.258** | -0.133ns | -0.098ns | 0.297** | 1.000 | | | | |
| LMC | 0.051ns | 0.072ns | -0.101ns | 0.001ns | 0.002ns | 0.005ns | 0.057ns | 1.000 | | | |
| RLER | -0.084ns | -0.082ns | -0.201ns | -0.201ns | 0.087ns | -0.403** | -0.291* | -0.104ns | 1.000 | | |
| LY | -0.079ns | -0.103ns | -0.407** | 0.018ns | 0.0139ns | -0.537** | -0.374** | -0.109ns | 0.433** | 1.000 | |
| FY | 0.713** | 0.891** | 0.661** | 0.561** | 0.509** | 0.440** | 0.156ns | 0.106ns | -0.067ns | 0.801** | 1.000 |

Note: different letters indicates significant difference, same letters indicates no significant difference

Table 8 shows different correlation results for various parameters. According to this table, there was a strong relationship between leaf area and total shoot length at significance level of 0.01 (0.868**). There was also a positive significant correlation between plant height and total shoot length which was 0.717**. Moreover, leaf thickness was also correlated significantly with total shoot length (0.632**). This result was almost the same for leaf number and total shoot length (0.626**). Leaf number was also correlated with leaf area and plant height which was significant at 0.01 level (0.734** and 0.521** respectively). Leaf area had a strong correlation with leaf thickness at significant level of 0.01 (0.805**). Leaf thickness and plant height together with leaf thickness and leaf number were significantly correlated which were 0.559 **and 0.653** respectively. There was a weak relationship between chlorophyll stability index and chlorophyll content at significant level of 0.01 (0.297**). Relative leaf expansion rate had negative medium relationships with chlorophyll (-0.403**) and chlorophyll stability index (-0.291*). Chlorophyll had medium relationship with leaf area, total shoot length, plant height, leaf number, and leaf thickness. Significant relationships were seen between leaf yield and chlorophyll content (-0.537**), leaf yield and chlorophyll stability index (-0.374**), and leaf yield and relative leaf expansion rate (0.433**). Finally, fruit yield was significantly correlated with TSL (0.713**), LA (0.891**), PH (0.661**), LT (0.561**), LN (0.509**), Chl (0.440**), and LY (0.801**).

**DISCUSSION**

Drought stress is one of the most imperative limiting factors for plant productions and yield. In this study, various drought stress levels were applied in order to evaluate morphological and physiological responses of strawberry plants to drought stress. Together with severity of stress and duration of drought stress, two different environments were performed to observe proper reactions of plants to stress. In the current investigation, natural condition performed the best leaf area result in comparison with protected environment. In both conditions, leaf area was reduced significantly when plants treated with 25% of soil moisture. On the other hand, plants treated with normal irrigation performed better results. In terms of duration of stress, in both environments, plant varieties were exposed to 60 days of drought stress and the result indicated that with each increase in time duration, leaf area was decreased (Fig. 1c). Varieties gave the best leaf area results in natural condition in all time duration compared to protected environment. The highest leaf area was observed in 10 days after drought treatment and 60 days duration showed the lowermost leaf area value in all varieties in both environments (Fig. 1c). These outcomes were similar to the results obtained by Rucker *et al*. (1995), who observed reductions in leaf area under drought stress. Treder (2008) also observed that drought stress decreased leaf area in various strawberry cultivars. Furthermore, Grant *et al*. (2010), Passioura (1996), and Rizza *et al*. (2004) reported the same results as obtained in this investigation.In the current research, shoot length was higher in natural condition compared to glasshouse environment (Fig. 2a). Plants produced the lowest shoot length in 25% of treatment in comparison with other treatments and those plants which were cultivated in normal condition performed the best rates (Fig. 2b). In terms of time duration, in both cultivation environments, all three varieties showed the lowermost and highest rates of shoot lengths when treated with 60 days and 10 days duration (Fig. 2c). All three varieties had higher shoot length in 10 days after drought stress and the rate was decreased by the passage of time in all varieties. The results obtained were in parallel with the studies performed by other researchers. According to Rizza *et al*. (2004), there are a lot of traits linked to water deficit stress like tiny plant size, early maturity, and diminished leaf area.In terms of plant height, all varieties showed the lowest height in protected condition in comparison with natural environments (Fig. 3a). Also, varieties had lower height when treated with 25% compared to 50% and normal irrigation (Fig. 3b). With each increase in duration of drought stress, plant height was reduced. 10 days duration showed the highest plant height compared with other duration (Fig. 3c). In another investigation conducted by Rizza *et al*. (2004), plant height was decreased under limited availability of water.In terms of leaf thickness, all varieties presented lower results in protected environment compared to natural conditions (Fig. 4a). Leaf thickness was affected negatively in all varieties treated with 25% soil moisture. Plants produced the lowest leaf thickness in 25% of treatment in comparison with other treatments and those plants which were cultivated in normal condition performed the best rates (Fig. 4b).In terms of time duration, there were fluctuations in leaf thickness from 10 to 60 days duration in both environments. However, after 40 days of treatment, plants started demonstrating their thinner leaves in glasshouse and natural conditions (Fig. 4c). Santacruz and Cock (1984) mentioned that reduction in leaf thickness can be occurred under drought stress conditions. Moreover, other scientists such as Ristic*et al*. (1991), Silva *et al*. (1999), and Sam *et al*. (2000) in their research in Zea mays, bean, and tomato observed thicker leaves under limited irrigation compared to normal availability of water. Moreno-Sotomayor *et al*. (2002) and Chandra *et al*. (2004) also detected thicker leaves under water stress in *Zea mays*. In terms of leaf number, all varieties presented lower results in protected environment compared to natural conditions (Fig. 5a). Leaf number was diminished by every decrease in soil moisture in which Bogota performed proper results in all treatments followed by Festival and Chandler (Fig. 5b). Plants produced the lower leaf number in 25% and 50% of treatments in comparison with control and those plants which were cultivated in normal condition performed the best rates (Fig. 5b).In terms of time duration, in both cultivation environments, there were increasing trends in terms of leaf number from 10 to 60 days duration in both natural and glasshouse conditions (Fig. 5c). When strawberry varieties were remained for 60 days under drought stress, leaf number was increased after 60 days of drought condition. Surprisingly, leaf number was lower in 10 days after drought stress, followed by 20, 30, 40, 50, and 60 days in all varieties and environments (Fig. 5c). It was predicted that leaf number would be less in 60 days compared to other time duration. Higher leaf number in 60 days could be related to environmental impact on the varieties as leaf number is mostly influenced by environment rather than genotype. Shao *et al*. (2008) claimed that the number of leaves per plant can be diminished by water stress. According to Grant *et al*. (2010), strawberry numbers of leaves were significantly reduced under the water deficit treatment. Chlorophyll activity is critical in drought resistance (Ghaderi and Siosemardeh, 2011). In the current research, it was observed that that all of the genotypes produced higher chlorophyll content in natural environment compared to glasshouse condition in which Chandler produced the highest chlorophyll content in both conditions (Fig. 6a). Plant varieties cultivated in natural condition performed better chlorophyll content results compared to protected environment in all soil moisture levels (Fig. 6b). The lowest results were observed when varieties treated with 25% and 50% of soil moisture in glasshouse and natural environments respectively. Varieties had the highest chlorophyll content under normal irrigation in both glasshouse and natural conditions. In terms of duration of stress, in both environments, plant varieties were exposed to 60 days of drought stress and the result indicated that with each increase in time duration, chlorophyll content was decreased (Fig. 6c). Varieties gave the best chlorophyll content results in natural condition in all time duration compared to protected environment. The highest chlorophyll content was observed in 10 days after drought treatment and 60 days duration showed the lowermost chlorophyll content in all varieties in both environments (Fig. 6c). The results obtained in this research were in agreement with the findings of Bradford and Hsiao (1982) and Chartzoulakis*et al*. (1993) indicating that chlorophyll content was diminished with each reduction in soil moisture levels. Chlorophyll content was declined during strong water stress in two varieties of strawberry (Ghaderi and Siosemardeh, 2011). In another study performed by Kirnak*et al*. (2001), chlorophyll content was reduced by 55% under drought stress conditions compared to control. The adverse effect of water stress on chlorophyll content has previously been shown for young peach trees by Steinberg *et al*. (1990). Drought stress affected strawberry plants chlorophyll stability which is one of the most important parameters. In the current investigation, Bogota and Chandler showed the highest and lowest chlorophyll stability index in two environments under drought stress (Fig. 7a). Plants produced the lowest and highest chlorophyll stability index in 25% and 50% of treatments in glasshouse and natural conditions respectively. Plants cultivated in normal condition performed the best rates (Fig. 7b). In terms of time duration, in both cultivation environments, all three varieties showed the lowermost and highest rates of chlorophyll stability index when treated with 60 days and 10 days duration (Fig. 7c). All three varieties had higher chlorophyll stability index in 10 days after drought stress and the rate was decreased by the passage of time in all varieties. These results were obtained as they were expected. According to Chaves *et al*. (2003), plant varieties respond to drought stress by severity and duration of stress in which duration of stress caused plants to produce lower values in this study. Leaf moisture content in one of the most imperative parameters demonstrating a better measurement for drought stress effect on plants. Varieties cultivated in glasshouse condition produced higher leaf moisture in which Bogota and Festival showed the lowest and highest plant height in two environments under drought stress (Fig. 8a). This result was not expected as it was supposed that plants in natural condition could have much more moisture content. This outcome obtained may be because of the impact of other environmental factors such as humidity, ventilation, diseases or nutrient concentrations. Plants produced the lowest leaf moisture content in 50% of treatment in comparison with other treatments and those plants which were cultivated in 25% condition performed the best rates (Fig. 8b). It was supposed that plants in 25% moisture levels could result in lower leaf moisture percentage. The result obtained in 25% soil moisture level was not similar to findings of Kirnak*et al*. (2001) who mentioned that leaf moisture content was negatively affected by various water stress levels. Varieties had better leaf expansion rates in natural condition compared to glasshouse. Chandler demonstrated better leaf expansion compared to other varieties while Bogota had higher expanded leaves in glasshouse condition which was negative (Fig. 9a). Plants were observed to have negative leaf expansion rate when treated with 25% and 50% drought treatments in both locations (Fig. 9b). Varieties cultivated in normal condition showed higher and positive expansion rate. All varieties had lower expansion rate in 25% drought treatment compared to 50% and control in both environments. Kirnak*et al*. (2001) observed that leaves expansion rates were decreased by water stress treatments in different eggplant varieties.Water deficit occurs when there is water limitation in the root area which results in reduced plant yield including vegetative and reproductive organs. Plants showed higher performance in terms of leaf yield in natural environment compared to glasshouse. Bogota performed the best leaf yield while Festival demonstrated the lowest rate in both locations (Fig. 10a). Leaf yield was higher in normal irrigation compared to 50% and 25% soil moisture levels respectively in both locations (Fig. 10b). Plants had the lowest yield in 25% treatment compared to 50% and control treatments.Fruit yield was higher in natural environment compared to glasshouse condition and plants showed lower fruit yield under stress conditions (Figs. 11aand 11b). These findings were similar to the results obtained by Li *et al*. (2010) in which strawberry yield was diminished as an outcome of soil moisture deficit. Klamkowski and Treder (2008) also observed variations among three strawberry varieties in root development and yield under drought stress.Correlation analysis was conducted to observe relationships of different traits affected by drought stress. It was observed that there were significant relationships between various traits with each other. Leaf area and total shoot length were correlated significantly and their relationships were strong. This indicates that with each reduction in leaf area, total shoot length was also decreased as there were positive relations. There was also a positive significant correlation between plant height and total shoot length indicating their strong correlation. Moreover, total shoot length was correlated significantly with leaf thickness (0.632**). This result was almost the same for leaf number and total shoot length (0.626**). This may be due to similar impacts of drought stress on both of these traits. Leaf number was also correlated significantly with leaf area and plant height and their correlations were strong and medium respectively. Leaf area had a strong correlation with leaf thickness (0.805**). Leaf thickness and plant height together with leaf thickness and leaf number were significantly correlated which were 0.559**and 0.653** respectively. Finally, leaf number was also correlated with leaf area and plant height which was significant at 0.01 level (0.734** and 0.521**, respectively). According to this table, there was a weak relationship between chlorophyll stability index and chlorophyll content at significant level of 0.01 (0.297**). There was also a weak correlation between leaf moisture content and chlorophyll which was not significant ($0.005^{ns}$). Relative leaf expansion rate had negative medium relationships with chlorophyll (-0.403**) and chlorophyll stability index (-0.291*). Significant relationships were seen between leaf yield and chlorophyll content (-0.537**), leaf yield and chlorophyll stability index (-0.374**), and leaf yield and relative leaf expansion rate (0.433**). Finally, fruit yield was significantly correlated with TSL (0.713**), LA (0.891**), PH (0.661**), LT (0.561**), LN (0.509**), Chl (0.440**), and LY (0.801**).

***Conclusion*:** Drought is an important limiting factor in many regions and drought stress has been restricting plants production and yield. Strawberry (*Fragariaananassa*L.) is one of the most imperative crops cultivated which is highly affected by this environmental obstacle. In this study, it was tried to evaluate different effects of drought stress regimes along with various duration of stress and different environments to get appropriate responses of strawberry plants to this limiting factor. From the results obtained, in all of the parameters, stress had negative effects on plants morphological and physiological responses in terms of plant height, leaf area, total shoot length, leaf number, leaf thickness, chlorophyll content, chlorophyll stability index, leaf moisture content, relative leaf expansion rate, leaf yield and fruit yield. All of these parameters and their values were reduced by severity and duration of stress. Furthermore, it was concluded that natural condition had much better performance in terms of all parameters compared to protected environment. Various scientists also confirmed the results obtained in this study. Future research should be focused more on drought stress and responses of different strawberry plants. Proper use of cultivation environment with appropriate light intensity, humidity, and ventilation should be considered to get good outcomes. Moreover, to improve validity and reliability of the research, more number of varieties should be chosen to observe different responses of plants and introduce the best variety under this limiting environment. Moreover, different growth stages and various sorts of plant genotypes including sensitive and tolerant ones can be selected to observe their performances under water stress limitations.

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