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# Pyramiding Descriptors Contributing Drought Stress Tolerance to Six Polyembryonic Rootstocks of Mango (*Mangifera indica* L.)

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Author KK carried out the experiment, data analysis, prepared the manuscript. Author VKS planning of experiment, supervision improvement of manuscript and final approval of the manuscript to be published. Author RD data analysis, graphical representation of data and review of the manuscript. Author AKP assisted in data recording, handling of instruments and improvement of manuscript. Author CAD review and improvement of manuscript. All authors read and approved the final manuscript.*

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## ABSTRACT

A better understanding of the drought stress on mango varieties is essential in an effort to keep the production and the quality of mango to meet the growing demand globally. Since drought stress has been one of the major abiotic stress affecting both production and quality of mango, we studied to understanding the drought stress phenomenon in mango, especially to evaluate the descriptors for drought stress tolerance in mango rootstocks. Irrigation was withhold for fifteen days in six polyembryonic mango rootstocks namely 'Goa', 'Vellaikulamban', 'Nekkare', 'Starch', 'Kitchner' and 'M 13-1' and then rehydrated up to five days. Morphological parameters, membrane stability index (MSI), wax content, chlorophyll content, gas exchange attributes were recorded at 0, 15$^{th}$ and 5$^{th}$ day of rehydration. MSI, chlorophyll content and gas exchange parameters such as intercellular $CO_2$, stomatal conductance, net photosynthesis, and transpiration rate were reduced while cuticular wax content increased in all the polyembryonic mango rootstocks under drought stress and after rehydration same were recovered back up to greater extent. Rootstock 'M 13-1' showed more capacity to recover under drought stress as compared to five others rootstocks of mango. Therefore, rootstock 'M 13-1' can be recommended for scion varieties for frequent drought prone areas for taking sustainable mango production.

*Keywords: Drought stress; mango rootstock; membrane stability index; chlorophyll content; wax content; gas exchange parameters.*

## 1. INTRODUCTION

Most of the climate change scenarios suggest an increase in the aridity in many areas around the world. Drought stress concomitant with high temperature and the radiations pose a preeminent environmental challenge to plant productivity and quality of the produce. Plant growth, dry mater and final commercial yield are significantly affected due to drought stress. However, time period, severity and speed have crucial roles during water deficit in crop plants (Anjum et al. 2011). Mango cultivation in India is hampered by various environmental challenges, *viz*., erratic bearing, mango malformation disease, alternate bearing, physiological disorders under erratic weather situations (Kumar et al. 2020). Several abiotic stresses such as extremes of temperature, drought, flooding, salinity and heavy metals are some of the other challenge to which plant are continuously exposed in the real filed conditions affecting the crop productivity and food sustainability worldwide (Waqas et al. 2019). Worldwide heat and drought stresses are projected to be more frequent, longer, and occurring earlier, which adversely affect the productivity of several crops including fruit crops. In the real filed condition plants are often exposed to variety of stress at a same time that can negatively affects the biochemical and physiological processes in plants (El-Basyoni et al. 2017). Changes in the patterns of precipitation and global warming-induced increase in evapo-transpiration rates have increased the frequency and severity of drought stress (Dai 2011).

There are three broad categories of drought tolerance mechanisms *viz*., drought avoidance, drought escape and biochemical tolerance during water stress (Plomion et al. 2016). The quantitative nature of drought and heat stress tolerance lowers the probability of developing genotypes with tolerance to such stresses via traditional breeding methods, which limits the success of this approach (Paulsen 2002). Plant growth and development, photosynthetic activity, membrane stability, pigment content, osmotic adjustment and leaf and soil water content, and productivity are significantly affected during water stress (Praba et al. 2009; Benjamin and Nielsen 2006). Depending on the plant species and their developmental stages, stress degree and severity, co-factors; the susceptible/ tolerance of plants to drought stress is determined (Demirevska et al. 2009). Acclimation of plants to water deficit is result of different events, which lead to adaptive changes in plant growth and physio-biochemical processes, such as growth rate, changes in plant structure, antioxidant defense system and osmotic potential of plant tissue (Duan et al. 2007).

Therefore, it is most important to explain the responses and adaptation of crop plants to drought stress and simultaneously takes actions for improving the ability of crop plants to drought stress. This may ensure optimum crop yields even under unfavorable abiotic stresses

conditions. Gas exchange attributes and chlorophyll fluorescence and related parameters are very crucial under abiotic stress especially drought and salinity stress. One of the first physiological responses of drought is inhibition of photosynthesis process in plant which is well observed in many crop plants [Cornic 1994; Lawlor 1995]. The prime factor of reduction of photosynthesis process due to drought stress is reduced carbon di-oxide ($CO_2$) diffusion from the atmosphere to the site of carboxylation in the leaf because of both a) stomatal closure and b) reduced mesophyll conductance (Chaves and Oleivira 2004; Grassi and Magnani 2005). The measurements of gas exchange parameters together with chlorophyll fluorescence and related attributes provide a good way to evaluate the photosynthetic performance in stressed plants (Jimenez et al. 1997) and also for providing clear understanding of behaviour of the photosynthetic machinery under drought stress (Maxwell and Johnson 2000).

Drought stress on agricultural land is increasing day by day and in many areas drought stress management is critical for the successful crop production. There is adequate genetic diversity exists within *Mangifera indica* (L.) which can be used for identification, selection and developing drought tolerant rootstock. However quantitative data are required with respect to critical limit of stress that mango trees can tolerate without optimal reduction in yield and fruit quality. The prospect for future cultivation of drought stress tolerant or resistant, high yielding genotypes of mango are very encouraging and a rapid and accurate method to identify drought tolerant mango seedlings for rootstock is urgently required. Therefore, present study was attempted to give a glimpse of descriptors combined with gas exchange parameters on the responses of six polyembryonic mango rootstock seedlings under induced drought stress.

## 2. MATERIALS AND METHODS

### 2.1 Site Description and Experimental Details

The experiment was conducted in the fruit physiology laboratory, Division of Crop Production, ICAR-Central Institute for Sub-tropical Horticulture, Lucknow (Uttar Pradesh), India. The Institute is geographically located at an elevation of 128 m above mean sea level in the indo-Gangetic plains at 26º85' N latitude and 80°90' E longitude in the middle part of Uttar Pradesh. During the period of study maximum and minimum temperatures were 43 and 15.5 $^0$C and maximum and minimum relative humidity was 96 and 34 %, respectively. There were six (two year old) polyembryonic mango rootstocks *viz*., 'Goa', 'Vellaikulamban', 'Nekkare', 'Starch', 'Kitchner' and 'M 13-1' collected from orchard and utilized for the study. The experiment was conducted in the net house covered with UV - stabilized green shade net 50 % absorbance made Net Lon. The pots were temporary covered with 50µ thick transparent polyethylene as rain shelter to protect from monsoon rains. Characteristics of the used pots soil was; pH 7.3, EC 0.02-0.38 dS/m, bulk density 1.4, field capacity 2.5 g/cm$^3$, water holding capacity 20.7%, porosity 43.9% *etc*. The present experiment was conducted in completely randomized block design replicated thrice and data were analyzed using online software (OPSTATE) developed by Sheoran et al. 1998.

### 2.2 Measurement of Morphological Parameters

Plant height was measured with the help of measuring scale from the base of the stem at the collar region to the tip of the terminal extension growth. The girth of the plant was measured at first node above the ground level with the help of Vernier caliper. The area of leaf was measured with help of Image Analyser software (Biovis Image Plus, Mumbai) and expressed in cm$^2$ and leaves were counted manually on the plants and expressed in numbers. The shape of leaves was visually observed and expressed as lanceolate/elliptical/ovate and new growth as leaf flushing on the plant was observed visually and expressed as present or absent.

### 2.3 Membrane Stability Index (MSI)

The membrane stability index (MSI) was estimated as described by Deshmukh et al. 1991. Leaf samples were cut into discs (1.88cm$^2$) of uniform size with the help of Cork Borer. These leaf discs were placed in test tubes containing 10 ml double distilled water in two sets. Then these test tubes were placed in a beaker containing water and placed in a water bath. One set was heated at 40°C for 30 minutes. Put out the test tubes from water bath and discarded the leaf discs and the electrical conductivity ($C_1$) of lichates was recorded. The second set was boiled at 100°C for 15 minutes and the electrical conductivity ($C_2$) of lichates was measured. Both conductivities were measured using a

conductivity meter LMCM 20 make Lamban Scientific Instruments Pvt. Ltd, Chennai, India. The membrane stability index was calculated by using the following formula:

$$MSI = [1 – (C1/C2)] \times 100$$

Where, $C_1$: electrical conductivity of leaf lichates at 40 $^0$C for 30 minutes, $C_2$: electrical conductivity of leaf lichates at 100 $^0$C for 15 minutes.

## 2.4 Cuticular Wax Content

Cuticular wax content in leaves was measured by the method according to Bewick et al. 1993 using chloroform. Leaf disc was taken with the help of Cork Borer. The leaf disc area (1.88cm$^2$) was measured with the help of Image Analyzer software (Biovis Image Plus, Mumbai). These leaf discs were immerged in 10 ml chloroform in glass vials for 10 minutes. After 10 minutes lids of vials were opened for evaporation of chloroform. Leaf discs were removed from vials after1 hour. Weight of vials was taken after fully evaporation of chloroform. The wax content was calculated and expressed as g/cm$^2$.

## 2.5 Chlorophyll Content

The chlorophyll was measured according to the method of Arnon 1994. The chlorophyll *a*, chlorophyll *b* and total chlorophyll contents in fresh leaves were determined by using double beam UV-VIS spectrophotometer make Decibel, Scientific Equipments, Model No. D.B.1261. Twenty five milli gram of leaf sample was crushed in 80 % acetone with the help of pestle and mortar. The well crushed solution filtered using Whatman 41 filter paper in 25 ml volumetric flask. The final volume of obtained extract was made up 25 ml with 80 % acetone. The volumetric flasks were covered with carbon paper and kept in dark. Absorbance of extract was measured at 663 and 645 nm against 80 % acetone as blank. The chlorophyll contents (g/l) were calculated using the following equations:

Chlorophyll *a* = 0.0127 x A 663- 0.00269 x A 645
Chlorophyll *b* = 0.0029 x A 663- 0.00468 x A 645
Total chlorophyll = 0.0202 x A 663 + 0.00802 x A 645

## 2.6 Estimation of Physiological Parameters

### 2.6.1 Gas exchange attributes

All the parameters were recorded before induction of drought stress (at 0 day) and 15$^{th}$ day of drought stress. Thereafter, all the plants were rehydrated by giving regular irrigation up to 5 days and gas exchange parameter were measured at 5$^{th}$ day after rehydration to know the recovery of the gas exchange parameters. Gas exchange parameter such as intercellular $CO_2$ concentration ($C_i$) net photosynthesis (A), stomatal conductance (gs), transpiration rate and associated parameters were recorded in fully developed 3$^{rd}$ or 4$^{th}$ mature leaf from the top with the help of Ciras- 3 Portable Photosynthesis System, make MA, USA fitted with air probe for minimizing the error of $CO_2$ effect. Three measurements were recorded in fully developed mature leaves from three plants of each rootstock. All the parameters were recorded with attached leaves to plant in their natural orientation from 9:30 to 11:00 am.

# 3. RESULTS

## 3.1 Effect of Induced Drought Stress on Morphological Characters

Increase in plant height (Table 1) was recorded in all the mango rootstocks being highest in rootstock ‘Goa’ (6.90 cm) and lowest in ‘M 13-1’ (0.17 cm) as compared to controlled plants. The increase in plant height varied from 0.24 to 11.66 %. While, there was significant increase in control plants of all the studied rootstocks and it ranged from 1.22 to 17.77 %. In control plants, slight increase in girth was observed in all the rootstocks except for ‘M 13-1’. In stressed plants, there was no increase in the girths size of the all rootstocks except in ‘Nekkare’ where nominal increase (0.13 cm) was observed. In rootstocks ‘Goa’, ‘Starch’, ‘Kitchner’ and ‘M 13-1’ average number of leaves increased *i.e*. 9.67, 9.66, 4.66 and 0.67, respectively; while in ‘Vellaikulamban’ (0.33) and ‘Nekkare’ (0.33) a reduction was observed. On the other hand, in control plants, number of leaves increased in all the rootstocks and increase was in number of 1.00 to 11.67. Leaf area was found reduced in all the rootstocks except ‘M 13-1’ as compared to control plants. Maximum reduction was measured in ‘Vellaikulamban’ (1.33 cm$^2$) and minimum in ‘Starch’ (0.19 cm$^2$). Increase in leaf area of rootstock ‘M 13-1’ was 0.70 cm$^2$. There were three kinds of leaf shapes in studied rootstocks. Lanceolate type was in ‘Goa’, ‘Starch’, ‘Kitchner’ and ‘M 13-1’ while, elliptical shape in ‘Vellaikulamban’ and ovate was in ‘Nekkare’. Two types of leaf margins were observed in studied rootstocks *i.e*. entire in Goa, Nekkare and Kitchner, while wavy in ‘Vellaikulamban’, ‘Starch’ and ‘M 13-1’(Table 1).

### 3.2 Effect of Induced Drought Stress on Membrane Stability Index (MSI)

Before drought stress, MSI was varied among all polyembryonic mango rootstocks and measured maximum (89.63 %) in rootstock 'Goa' and minimum (78.52%) in 'Vellaikulamban' (Table 2). After 15 days of induced drought stress MSI was reduced in all the mango rootstocks and noticed maximum (78.40%) in 'Starch' followed by 'M 13-1' (77.38%) and minimum (61.71%) in Kitchner. As for as reduction in MSI among mango rootstocks is concerned, maximum reduction (23. 65 %) was recorded in rootstock 'Goa' followed by 'Kitchner' (21.40%) and minimum (4.40 %) in 'M 13-1'. After 5 days of rehydration, MSI was reached highest 81.22% in 'Starch' followed by 80.65% in 'M 13-1' and lowest 72.19% in 'Nekkare' while, maximum recovery (98.87 %) of MSI was noted in rootstock 'M 13-1' followed by Starch (97.91%) and minimum (89.81 %) in 'Nekkare' (Fig. 1).

### 3.3 Effect of Imposed Drought Stress on Cuticular Wax Content

Cuticular wax content (Table 2) in leaves of all six mango rootstocks ranged from 7.26-16.67 mg/cm$^2$ before imposing drought stress. It was significantly increased after 15 days of induced drought stress in all the rootstocks. Wax content was measured maximum (46.43 mg/cm$^2$) in 'M 13-1' followed by 'Nekkare' (44.83 mg/cm$^2$) and minimum was in 'Kitchner' (35.03 mg/cm$^2$). Similarly, maximum increase in wax content among all mango rootstocks after drought stress was observed in 'M 13-1' (39.17 mg/ cm$^2$) followed by 'Nekkare' (28.16 mg/cm$^2$) while minimum was in 'Kitchner' (14.00 mg/ cm$^2$) (Fig. 2). Likewise, five days after rehydration wax recovery was reached back at highest in 'M 13-1' (6.19 mg/cm$^2$) followed by 'Goa' (6.79 mg/cm$^2$) and lowest in 'Kitchner' (5.72 mg/cm$^2$) (Table 2).

### 3.4 Effect of Induced Drought Stress on chlorophyll Content

Before drought stress chlorophyll *a* and *b* in six mango rootstocks (Figs. 3 and 4) varied from 0.79 (Kitchner) to 1.81 mg/g (Starch) and 0.060 (Kitchner) to 0.137 mg/g (Starch), respectively. Induced drought stress reduced the chlorophyll *a* content in all rootstocks and measured lowest chlorophyll *a* in 'Kitchner' (0.15 mg/g) which was at par with 'Vellaikulamban' (0.17 mg/g) and recorded highest in 'M 13-1' (0.51 mg/g). After rehydration, recovery of chlorophyll *a* was maximum (0.67 mg/g) in mango rootstock 'M 13-1' and minimum was in 'Starch' (1.03 mg/g) (Fig. 3). Similar pattern were also measured for chlorophyll *b* in studied mango rootstocks (Fig. 4). After induced drought stress, chlorophyll *b* was noted maximum (0.043 mg/g) in 'Starch' and minimum in 'Nekkare' (0.015 mg/g). Five days after rehydration, chlorophyll *b* recovery was also found highest (0.057 mg/g) in rootstock 'M 13-1' and lowest was in 'Starch' (0.050 mg/g).

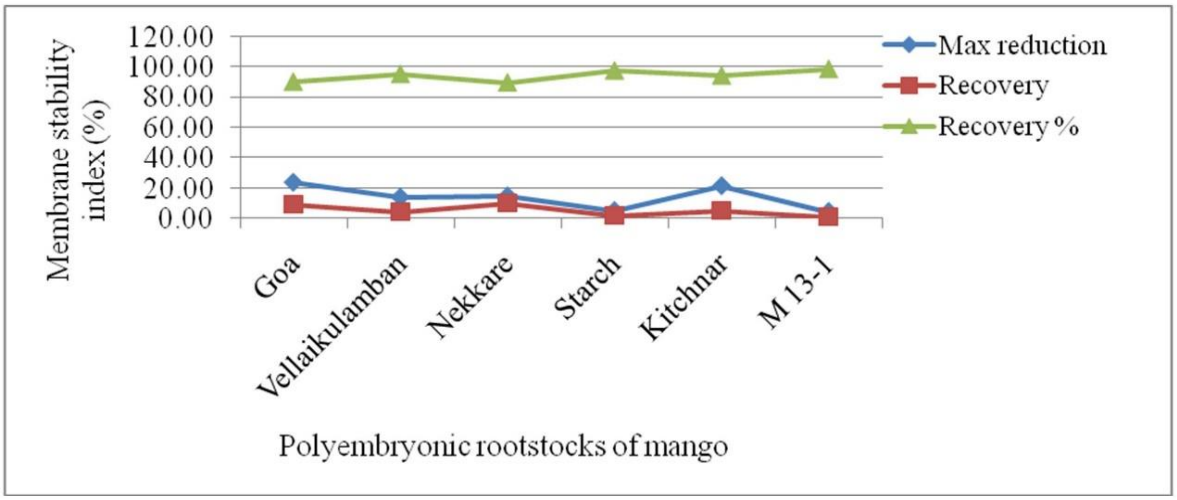


**Fig. 1. Membrane stability index in mango rootstocks on 15$^{th}$ day of drought stress and recovery after 5 days of rehydration**

**Table 1. Effect of induced drought stress on morphological parameters of polyembryonic rootstocks of mango**

| Rootstock | Rootstock height (cm) | | Rootstock girth (cm) | | Number of leaf/ plant | | Leaf area ($cm^2$) | | Leaf shape | | Leaf margin | | New growth | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Zero day | At 15th day | Zero day | At 15th day | Zero day | At 15th day | Zero day | At 15th day | Zero day | At 15th day | Zero day | At 15th day | Zero day | At 15th day |
| Goa | 59.17 | 66.07 | 1.20 | 1.20 | 34.00 | 43.67 | 63.85 | 63.62 | Lanceolate | Lanceolate | Entire | Entire | Present | Present |
| Vellaikulamban | 64.00 | 66.17 | 1.40 | 1.40 | 23.33 | 23.00 | 170.47 | 169.13 | Elliptical | Elliptical | Wavy | Wavy | Absent | Present |
| Nekkare | 48.10 | 52.13 | 1.00 | 1.13 | 22.67 | 22.33 | 140.91 | 140.61 | Ovate | Ovate | Entire | Entire | Absent | Present |
| Starch | 52.67 | 55.00 | 1.40 | 1.40 | 21.00 | 30.67 | 50.90 | 50.70 | Lanceolate | Lanceolate | Wavy | Wavy | Present | Present |
| Kitchner | 79.17 | 82.00 | 1.70 | 1.70 | 42.33 | 47.00 | 192.21 | 191.90 | Lanceolate | Lanceolate | Entire | Entire | Present | Present |
| M 13-1 | 69.50 | 69.67 | 1.40 | 1.40 | 27.67 | 28.33 | 72.24 | 72.94 | Lanceolate | Lanceolate | Wavy | Wavy | Absent | Absent |
| CD at 5% | 6.05 | 10.47 | 0.29 | N/A | 3.69 | 5.70 | 5.74 | 4.18 | --- | --- | --- | --- | --- | --- |
| SEm± | 1.94 | 3.36 | 0.09 | 0.09 | 1.18 | 1.83 | 1.84 | 1.34 | | | | | | |
| Control plants | | | | | | | | | | | | | | |
| Goa | 62.83 | 74.00 | 1.10 | 1.23 | 44.67 | 46.00 | 73.53 | 74.05 | Lanceolate | Lanceolate | Entire | Entire | Present | Present |
| Vellaikulamban | 60.00 | 70.10 | 1.00 | 1.20 | 17.33 | 23.67 | 164.64 | 164.83 | Elliptical | Elliptical | Wavy | Wavy | Absent | Absent |
| Nekkare | 53.50 | 57.17 | 1.07 | 1.27 | 22.00 | 23.00 | 166.59 | 166.86 | Ovate | Ovate | Entire | Entire | Present | Present |
| Starch | 39.83 | 38.33 | 1.03 | 1.23 | 13.00 | 14.33 | 84.94 | 85.22 | Lanceolate | Lanceolate | Wavy | Wavy | Present | Present |
| Kitchner | 63.67 | 65.00 | 1.47 | 1.53 | 37.33 | 49.00 | 174.17 | 174.31 | Lanceolate | Lanceolate | Entire | Entire | Present | Present |
| M 13-1 | 103.73 | 105.00 | 1.80 | 1.80 | 42.67 | 44.33 | 45.59 | 46.22 | Lanceolate | Lanceolate | Wavy | Wavy | Absent | Absent |
| CD at 5% | 7.42 | 6.81 | N/A | 0.15 | 3.76 | 3.44 | 4.13 | 3.62 | --- | --- | --- | --- | --- | --- |
| SEm± | 2.38 | 2.18 | 0.08 | 0.04 | 1.21 | 1.10 | 1.32 | 1.16 | | | | | | |

**Table 2. Effect of induced drought stress on membrane stability index and cuticular wax content on 0 day, 15th day of drought stress and 5th day after rehydration in six polyembryonic rootstocks of mango**

| Rootstock | Membrane stability index (%) | | | Cuticular wax content (mg/cm²) | | |
|---|---|---|---|---|---|---|
| | 0 day | 15th day | 5th day of rehydration | 0 day | 15th day | 5th day of rehydration |
| Goa | 89.63 | 65.98 | 79.98 | 13.83 | 38.6 | 6.79 |
| Vellaikulamban | 78.52 | 64.66 | 74.03 | 16.43 | 42.6 | 6.04 |
| Nekkare | 82.38 | 67.68 | 72.19 | 16.67 | 44.83 | 6.21 |
| Starch | 83.31 | 78.40 | 81.22 | 16.03 | 42.47 | 5.92 |
| Kitchner | 83.11 | 61.71 | 77.68 | 21.03 | 35.03 | 5.72 |
| M 13-1 | 81.78 | 77.38 | 80.65 | 7.26 | 46.43 | 6.19 |
| CD at 5% | 2.45 | 2.66 | 5.56 | 0.035 | 0.108 | 0.174 |
| SEm± | 0.78 | 0.85 | 1.78 | 0.011 | 0.035 | 0.056 |

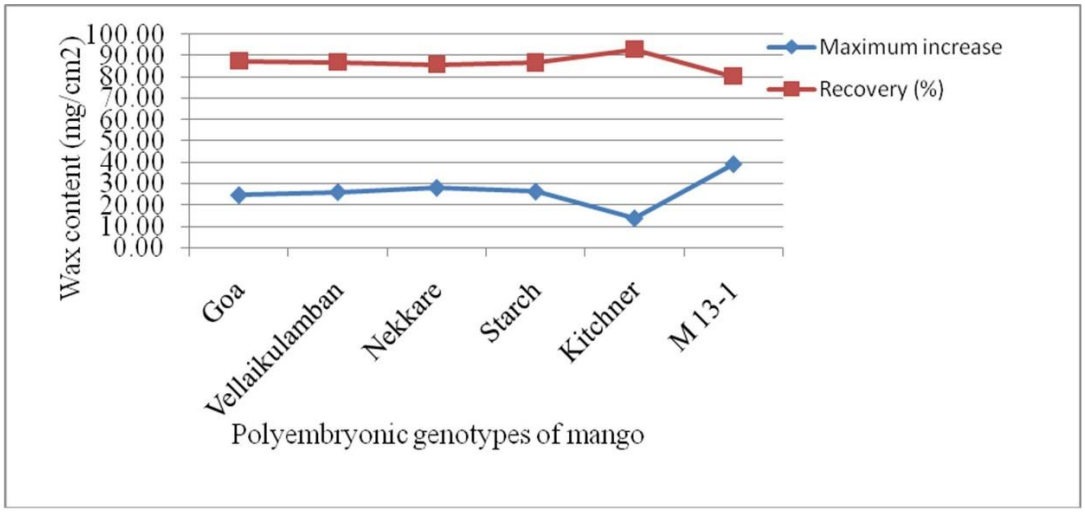


**Fig. 2. Cuticular wax in leaves of mango rootstocks on 15th day of drought stress and recovery after 5 days of rehydration**

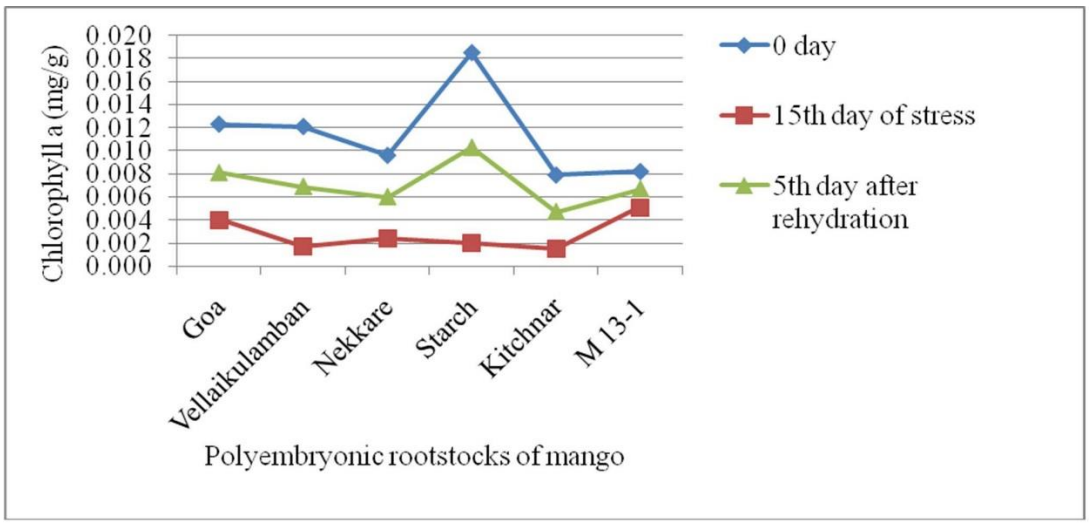


**Fig. 3. Chlorophyll *a* content in mango rootstocks after drought stress and rehydration**

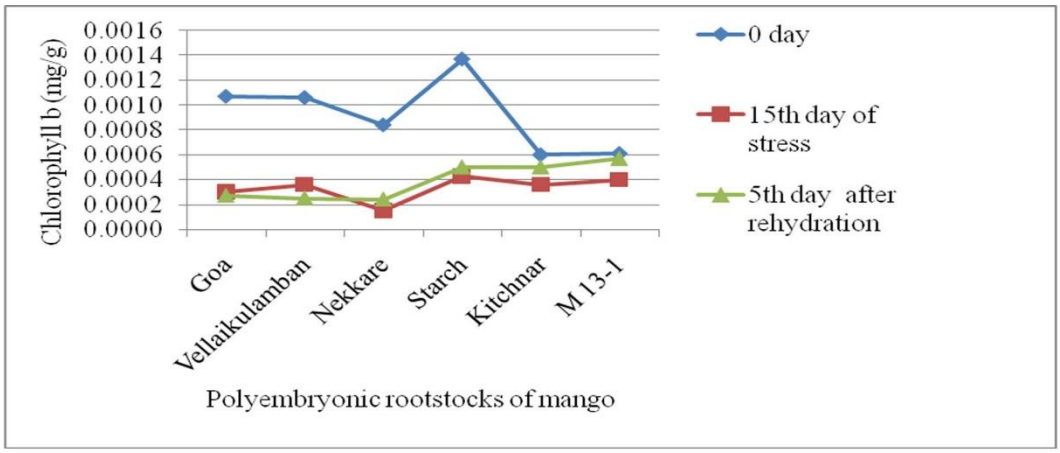


**Fig. 4. Chlorophyll *b* content in mango rootstocks after drought stress and rehydration**

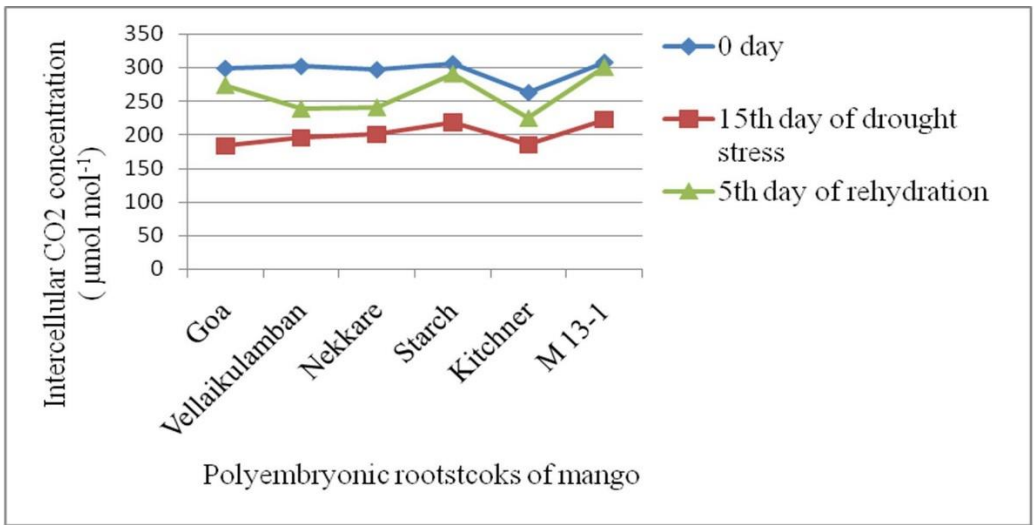


**Fig. 5. Effect of drought stress on intercellular $CO_2$ concentration of mango rootstocks**

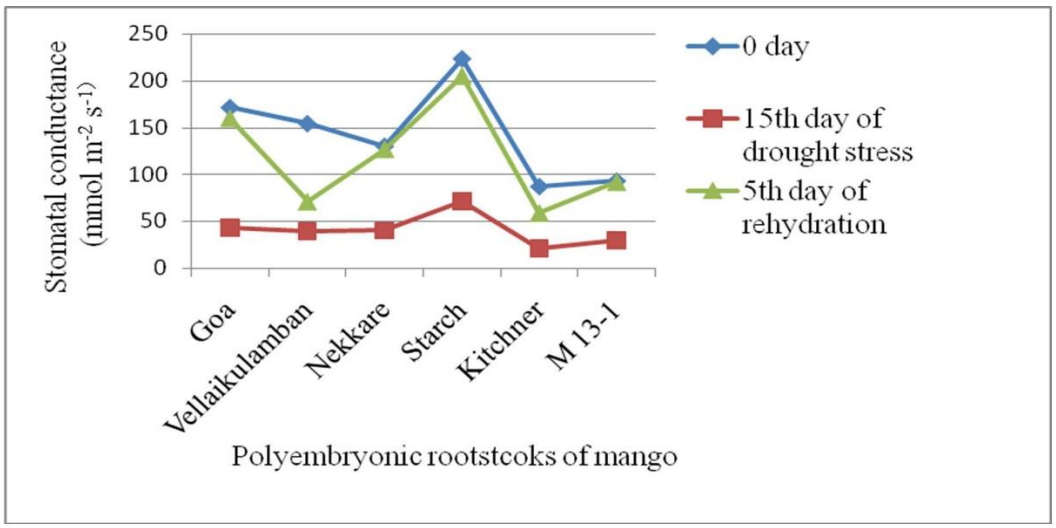


**Fig. 6. Effect of drought stress on stomatal conductance of mango rootstocks**

### 3.5 Effect of Induced Drought Stress on Gas Exchange Parameters

Gas exchange parameters such as intercellular $CO_2$ concentration (*Ci*), stomatal conductance (*gs*), net photosynthesis (*A*), transpiration rate (*E*) *etc* were measured before and after imposing drought stress in all six polyembryonic mango rootstocks. Gas exchange rates varied among these rootstocks (Figs. 5, 6, 7 and 8). Before drought stress (at 0 day) Ci was measured maximum (307 µmol $mol^{-1}$) in 'M 13-1' followed by 'Starch' (305 µmol $mol^{-1}$) and 'Vellaikulamban' (301 µmol $mol^{-1}$) while minimum (262 µmol $mol^{-1}$) in 'Kitchner'. Highest stomatal conductance was noted in 'Starch' (223 mmol $m^{-2}$ $s^{-1}$) followed by 'Goa' (171 mmol $m^{-2}$ $s^{-1}$) and lowest in 'Kitchner' (87 mmol $m^{-2}$ $s^{-1}$). Likewise, net photosynthesis was observed maximum (10.40 µmol $m^{-2}s^{-1}$) in rootstock 'Starch' and minimum (4.70 µmol $m^{-2}s^{-1}$) in 'Kitchner'. Transpiration rate was measured highest in rootstock 'Starch' (4.02 mmol $m^{-2}$ $s^{-1}$) and lowest (2.08 mmol $m^{-2}$ $s^{-1}$) in 'M 13-1' before imposition of drought).

### 3.6 Intercellular $CO_2$ Concentration (CI) and Stomatal Conductance (GS)

There was significant reduction noticed in all the polyembryonic mango rootstocks with respect to both *Ci* and *gs* after 15 days of drought stress. After drought stress, lowest Ci (183 µmol $mol^{-1}$) was recorded in 'Goa' which was at par with 'Kitchner' (184 µmol $mol^{-1}$)' followed by 'Vellaikulamban' (195 µmol $mol^{-1}$) and highest (222 µmol $mol^{-1}$) in 'M 13-1'. Five days after rehydration, maximum recovery (301µmol $mol^{-1}$) was noted in 'M 13-1' while minimum in 'Vellaikulamban' (269 µmol $mol^{-1}$). Similarly, (at 0 day) lowest *gs* (87 mmol $m^{-2}$ $s^{-1}$) was recorded in 'Kitchner' followed by 'M 13-1' (93 mmol $m^{-2}$ $s^{-1}$) and highest (223 mmol $m^{-2}$ $s^{-1}$) in rootstock 'Starch' followed by 'Goa' (171 mmol $m^{-2}$ $s^{-1}$). Similar pattern was also measured after drought stress and lowest *gs* (21 mmol $m^{-2}$ $s^{-1}$) was recorded in rootstock 'Kitchner' and highest in 'Starch' (71 mmol $m^{-2}$ $s^{-1}$). After rehydration, maximum recovery of stomatal conductance (92 mmol $m^{-2}$ $s^{-1}$) was found in 'M 13-1' followed by 'Nekkare' (127 mmol $m^{-2}$ $s^{-1}$) and minimum in 'Vellaikulamban' (71 mmol $m^{-2}$ $s^{-1}$) (Figs. 5 and 6).

### 3.7 Net Photosynthesis (A) and Transpiration Rate (E)

Before drought stress, net photosynthesis was observed maximum (10.40 µmol $m^{-2}s^{-1}$) in mango rootstock 'Starch' followed by 'Nekkare' (7.90 µmol $m^{-2}s^{-1}$) and minimum (4.70 µmol $m^{-2}s^{-1}$) in 'Kitchner'. Likewise, after induced drought stress, *A* was reduced significantly in all the rootstock varieties and measured highest (5.10 µmol $m^{-2}s^{-1}$) in 'Starch' and lowest (1.40 µmol $m^{-2}s^{-1}$) in 'Kitchner'. After rehydration, *A* was recovered maximum (4.60 µmol $m^{-2}s^{-1}$) in mango rootstock 'M 13-1' followed by Nekkare (7.40 µmol $m^{-2}s^{-1}$) and minimum recovery was measured in 'Starch' (8.70) followed by 'Vellaikulamban' (3.50 µmol $m^{-2}s^{-1}$) (Fig. 7). Similar to *A*, transpiration rate was also recorded highest in rootstock 'Starch' (4.02 mmol $m^{-2}$ $s^{-1}$) and minimum in 'M 13-1' (2.08 mmol $m^{-2}$ $s^{-1}$) before induction of drought stress. After drought stress, *E* was found lowest (1.13 mmol $m^{-2}$ $s^{-1}$) in 'M 13-1' followed by 'Kitchner' (1.18 mmol $m^{-2}$ $s^{-1}$) and highest in 'Starch' (2.30 mmol $m^{-2}$ $s^{-1}$). After rehydration, mango rootstock 'M 13-'1 was recovered maximum *E* (1.91 mmol $m^{-2}$ $s^{-1}$) followed by 'Goa' (2.76 mmol $m^{-2}$ $s^{-1}$) and minimum recovery was found in 'Vellaikulamban' (1.94 mmol $m^{-2}$ $s^{-1}$) (Fig. 8).

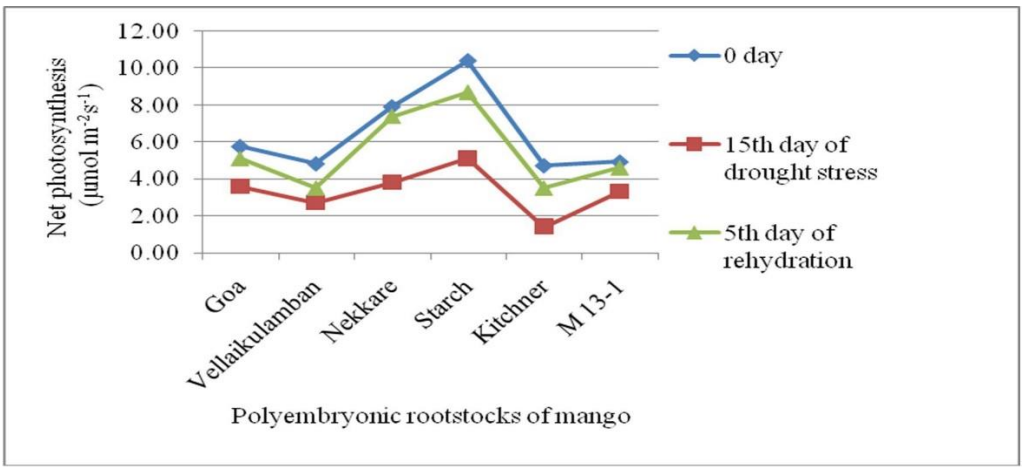


**Fig. 7. Effect of induced drought stress on net photosynthesis of mango rootstocks**

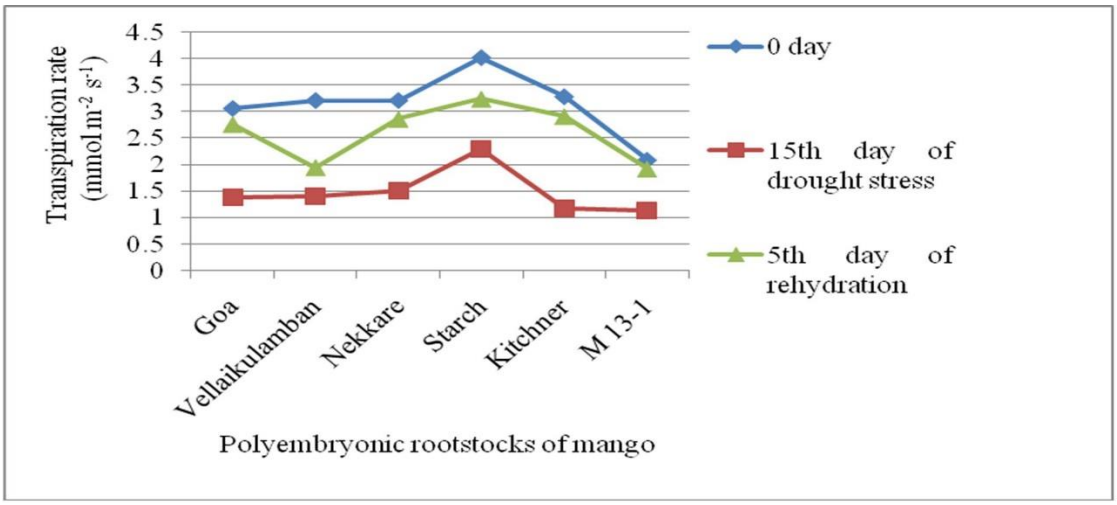


**Fig. 8. Effect of drought stress on transpiration rate of mango rootstocks**

## 4. DISCUSSION

Plants cope up with drought stress conditions through modifications in morphological, physiological, biochemical and anatomical traits (Lawson and Leakey, 2024). Primary response by the plants under drought stress is to minimize stress, by preventing the accumulation of fluids or harmful ions in sensitive leaf tissues. In case of mild or short duration stress, alone avoidance mechanisms may be enough to sustain plant's performance (Ziogas et al. 2021). Drought progressively decreases $CO_2$ assimilation rates due to reduced stomatal conductance. Stress reduces plant's growth attributes by disturbing leaf and soil water contents and water use efficiency. It disrupts photosynthetic pigments and reduces the gas exchange leading to reduction in plant growth and productivity (Anjum et al. 2011). Closure of stomata is a key avoidance mechanism in plants that works under short term drought. However, in long duration stress, reduces leaf biomass, leaf rolling flexibility, increasing cuticular waxes, increasing the root/shoot ratio by creating a deeper and thicker root system and regulating root water conductivity (Ziogas et al. 2021). In the present study, imposed drought stress has affected significantly morphological, physiological as well as gas exchange attributes more or less in all the polyembryonic mango rootstocks.

Drought stress, temporary or permanent, severely hampers the growth and developmental processes in plant more than any other abiotic stress (Anjum et al. 2011). Slight increase was measured in plant height of mango rootstocks after drought stress as compared to control plants where better increase was observed. Nominal increase in plant girth was noticed in rootstock 'Nekkare' while in others no increase was measured. An interesting feature was seen in case of number of leaves per plant due to drought stress in studied mango rootstocks. Increment in leaf numbers was found in four rootstocks *i.e.* 'Goa', 'Starch', 'Kitchner' and 'M 13-1; while decrease number was observed in 'Vellaikulamban' and 'Nekkare'. Significant reduction in leaf area was measured in all the mango rootstocks except 'M 13-1'. The slight increase in heights in studied mango rootstocks might be due to short duration of water stress condition. Another possible reason may be due to inbuilt tolerance mechanism to water stress in mango rootstock which was also earlier reported by several workers in many crops plants including fruit crops (Demirevska et al. 2009; Duan et al. 2007, Cameron et al. 2006 and Solanki 2015). Water deficits reduce the number of leaves per plant and individual leaf size, leaf longevity by decreasing the soil's water potential. Reduction in leaf area due to drought stress attributed to suppression of leaf expansion by reducing photosynthetic machinery. Expansion of leaf area mainly depends on assimilate supply, leaf turgor pressure and temperature (Rucker et al. 1995). In mango appearance of vegetative flushes is greatly reduced during water stress period. The water stress also causes reduction in number of leaves in a flush, the flush length, and leaf water contents (Perera-Castro et al., 2023; Laxman and Bhatt 2017). Reducing the shoot growth in grape (Hardie and Martin 2000), water-deficit stress treatment caused 50% reduction in leaves of papaya (Masri et al. 1990). Khan *et al*. 2001 concluded that plant height, stem diameter,

leaf area decreased noticeably with increasing water stress. Drought stress reduced plant height by declining cell enlargement and rapid leaf senescence process (Manivannan et al. 2007).

Though several factors affects membrane integrity, this parameter would definitely provide some insight to screen out the mango rootstocks and scion. MSI was found ranged from 78.52 to 89.63 % among studied mango rootstocks. Ravishankar et al. (2012) also reported similar results in mango and they concluded varied MSI in mango genotypes in the range of 58-86% under open control conditions. Induced drought stress caused reduction in membrane stability index among all mango rootstocks. The results are in the close conformity with the findings of decline in the MSI due to drought stress by Dubey et al. (2023) and Ravishankar et al. (2012) in mango genotypes. In the present study, after rehydration MSI had recovered highest and quickly in rootstock 'M 13-1' and lowest recovery was in 'Nekkare'. Thus, the rootstock 'M 13-1' had more capacity to recover with drought as compared to other rootstocks. Therefore, it is very clear that membrane stability index varied among the rootstock varieties under drought stress condition. Long term study on this line would be useful to make one of the important drought stress parameters for indexing the tolerant rootstock for drought stress in mango.

Deposition and composition of wax affects water deficit tolerance and ultimately final produce in plants. Cuticular waxes are active components which play a key role for adaptation to biotic and abiotic stresses in plant (Lihavainen et al., 2018). During water stress, stomata close and cuticular transpiration has significant importance (Cameron et al. 2006; Zhu et al., 2024). In the present study, there were significant differences among all rootstocks with respect to cuticular wax content in leaves which were varied from 7.26-16.67 mg/cm$^2$. An increase in cuticular wax due to drought stress was noted in all the mango rootstocks being maximum increment in rootstock M 13-1 and minimum was in Kithcner. However, after watering it was recovered highest in mango rootstock 'M 13-1' followed by 'Goa' and lowest in 'Kitchner'. As an increase of cuticular wax synthesis during water stress has been reported in several plants such as tree tobacco (Cameron et al. 2006) and sesame (Kim et al. 2007) and they had proposed an active role of cuticle in preventing plant desiccation. Cuticular waxes are protecting barrier of water loss. Thus, plant maintaining water by reducing cuticular water loss and this serves as an adaptation mechanism to drought. Additionally, Solanki and Sarangi (2015) had studied induced drought stress for 7 days and then rehydrated up to 7 days in two genotypes of peanut. They had reported increase in wax load in leaves with the increasing in the intensity of drought stress. However, the increase in wax content in K-9 genotype was found significantly higher than JL-24 genotype.

Chlorophyll is essentially required for photosynthesis in plants. Chlorophyll content in leaves has positive relation to photosynthetic rate. Both the chlorophyll a and b are prone to soil dehydration (Farooq et al. 2009). During water deficit period, photosynthesis is hampered due to reduction in chlorophyll contents in plant leaf. Drought stress induced decline in chlorophyll due to reduction in chloroplast membranes, excessive swelling and the appearance of lipid droplets (Kaiser et al. 1981). In the present study, induced drought stress showed declined trend in all the mango rootstocks for both chlorophyll a and b content. Maximum reduction of chlorophyll a and b content were recorded in rootstocks 'Kitchner' and 'Nekkare', respectively while minimum was in 'M 13-1'. However, quick and highest recovery of chlorophyll was measured in rootstock 'M 13-1' after rehydrating soil up to five days. Reduced chlorophyll contents due to water stress may be directly correlated with decline in the photosynthetic potential by affecting carbon assimilation process in the plant. During water stress, leaves started decreasing green colour which may degrade chlorophyll content by prevention of its biosynthesis. Present results are also supported by the findings of Faria-Silva and Siva (2023) and Faria-Silva et al. (2020) and in both studied they had concluded that drought stress caused reduction in the chlorophyll a, b and total content in mango. Therefore, from a physiological point of view, leaf chlorophyll content is a descriptor of significant interest while selecting drought tolerant/susceptible varieties.

The nature of the stress was of great importance in the water relation of the trees under conditions of high crop evapo-transpiration, since the trees showed difference responses under drought stress compared with the control. These differences could be due to the fact that mango trees have some tolerance-avoidance mechanisms and low transpiration, less fluctuation in relative water content to maintain their water status during soil water deficit (Singh et al. 2011 and Singh et al. 2010). Drought stress declines gas exchange parameters such as net photosynthesis, transpiration rate, stomatal

conductance, water use efficiency and intercellular $CO_2$ as compared to well watered control plants. There were more or less differences found among six rootstocks for gas exchange parameters before imposing drought stress in the present study. After water deficit, gas exchange attributes significantly reduced in all the mango rootstocks and recovered back after rehydration up to great extent as per tolerance-avoidance mechanisms of particular rootstock variety. There was reduction of *Ci* and *gs* noticed in all six mango rootstocks after drought stress as compared to well watered plants. Further, after rehydration, maximum recovery of these parameters was measured in polyembryonic mango rootstock 'M 13-1' and minimum was in 'Vellaikulamban'. The results were obtained by Luvaha et al. (2008) in mango with deficit irrigation; Ma et al. (2006) in pears-jujube and Arbona et al. (2005) in citrus also coinciding with the present study.

Similarly, reduction in net photosynthesis and transpiration rate were also recorded in all mango rootstock treatments as compared to irrigated plants. In response to drought, one of the most sensitive indicators of plant's over all physiological state is stomatal behaviour and transpiration. Stomatal closer permits the plant to balance water loss with carbon uptake (A), in turn improving long term water use efficiency and survival. Therefore, reduction in transpiration rate is an important physiological descriptor of water deficit in plant. Reduce carbon uptake caused by stomatal closer would account for reduction in the rate of photosynthesis recorded in all varieties after imposition of drought being maximum recovery was obtained in 'M 13-1' after rehydration. The results of present experiment were coinciding with the findings of several other workers in mango (Luvaha et al. 2008; El-sheery and Cao 2008; Santos et al. 2013; Helalya et al. 2017). This reveals that studied mango rootstocks had different levels of physiologically drought tolerance-avoidance mechanism. Moreover, the variation among rootstocks for most of the described descriptors might be due to their level of tolerance/resistance mechanism against drought stress conditions. There are several mechanisms *viz*., plant morphology, changes in growth pattern, defense mechanisms *etc*. by which plants adapted against drought stress conditions (Zandalinas et al. 2018).

## 5. CONCLUSIONS

There are several morphological, biochemical, physiological and molecular mechanisms involved in drought stress tolerance in plants. Different experimental trials have been conducted in recent past which clearly demonstrated that tolerant genotypes/rootstocks can be used as a solid solution to mitigate the ill effect of drought stress to sustain the productivity. Water stress immediately after fruit set in mango increases fruit drop. So, protective watering is required during the fruit development period. Thus, use of tolerant rootstocks is very essential for realizing sustainable yield under water-limiting conditions. Therefore, the quick adaptation strategy may be switching over to drought tolerant cultivars by grafting susceptible commercial cultivars onto tolerant mango rootstocks in frequent drought prone areas. However, long term study on this line would be useful to pyramiding traits to drought stress for indexing the tolerant polyembryonic mango rootstocks for sustainable yield.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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