Biochemical responses of monoembryonic and polyembryonic seedlings of mango rootstocks under salt stress conditions

Jagan Singh Gora, Ramesh Kumar, Chet Ram and VK Singh

Abstract
The present investigation was carried out to study the biochemical response of monoembryonic and polyembryonic rootstocks seedlings to saline irrigation water. Two rootstocks, Dashehari and Bappakai seedlings were subjected to different NaCl concentration (0, 15.8, 31.6, 37.48 and 74.97 g NaCl/10 kg pot soil). The result indicated that proline content was increased with enhanced salinity level and recorded the highest 0.042 and 0.033 µg/mg with 74.9 g NaCl salinity level in monoembryonic and polyembryonic rootstocks respectively, while total chlorophyll and carotenoids were decreased with increasing salinity level. Reducing sugar and total sugar increased from 0.003 to 0.010 percent in monoembryonic and 0.003 to 0.014 percent in polyembryonic with increasing salinity level from 0 to 74.9 g per pots, whereas starch content decreased in both type embryonic rootstocks. The polyembryonic seedlings was found more tolerant to NaCl by responding increased proline and sugar content, with low decline in chlorophyll and carotenoid content. Thus, polyembryonic seedlings proved to be the more adaptable rootstock to saline conditions.

Keywords: Monoembryonic, polyembryonic, rootstocks Dashehari, Bappakai

Introduction
The mango (Mangifera indica L.) is one of the most important fruit crops at the global level, especially in Asia. Its popularity and importance realized by the fact that it is often referred as ‘King of fruits’ in the tropical world (Singh, 1996) [43]. Among fruits, mango fruit is the richest source of vitamin A (4800 IU) and also contain amino acids, carbohydrates, fatty acids, minerals, organic acids and proteins (Ara et al., 2014; Shobana and Rajalakshmi 2010; Mathew 1983 and Albert 1984). India is the leading producer of mango in the world, contributing more than 40.48% of the total world production (FAO, 2010). Mango occupies the first position of fruit acreage, but its yield is very low as compared to other horticultural crops. Mango seedlings is one of the most sensitive to stress conditions such as salinity, drought, heat, flooding and heavy metal toxicity etc., where the various anthropogenic activities have accentuated the existing stress factors (Allakhverdiev et al., 2000; Siringam et al., 2012) [3, 44]. Among these stresses, salinity stress is a serious problem in mango cultivation worldwide (Yildirim et al., 2009; Qin et al., 2010) [49, 35].

Salt concentrations in irrigation water affects physiological and biochemical processes negatively including water relations and gas exchange attributes (Maeda and Nakazawa, 2008) [27], nutritional imbalance (Yang et al., 2008) [48], disturbing the membranes stability (Dogan et al., 2010) [14]. It has adverse effect on leaf chlorophyll and relative water content (RWC), as reported in S. argentea seedlings due to salt stress (Qin et al., 2010). The high salt concentrations cause a great reduction in growth parameters such as fresh and dry weights of shoots and roots (Balal et al., 2011) [10]. Mango is well known very sensitive crop to saline conditions (Maas, 1986), leading to scorched leaf tips and margins, leaf curling, and in severe cases reduced growth, abscission of leaves, and death of trees (Gora et al., 2017 and Jindal et al., 1976) [18], information concerning the salt tolerance of mango rootstocks is lacking, particularly on the impact of salinity on fruit yield (Ayers and Westcot 1989; Maas and Grattan, 1999). Mango seeds can be classified into two groups, monoembryonic and polyembryonic, based on their mode of reproduction.
Monoembryonic mango seed contain a single zygotic embryo, and hence only one seedling per seed, that is probable hybrid origin (Litz et al., 1984, and Patena et al., 2002) [26, 31]. Polyembryonic mango seeds can contain one or more embryos; one is usually, but not always zygotic (Litz 1984 and Dominguez et al., 2004) [20]. Adventitious embryos develop from the nucellus, a maternal tissue surrounding the embryo sac, and consequently the seedlings of polyembryonic mangoes are usually very similar to the maternal parent.

The polyembryonic mango has a greater salt tolerance than mono embryonic population (Kadman et al., 1976) [24]. Poly embryonic mango mostly have poor fruit quality and have little commercial value but they have potential to be used as a root stock for saline/alkaline soil or when irrigation water contains excess sodium or other soluble injurious ions for plants. The salt tolerant rootstocks M-13-1 and Gomera-1, bears capacity to restrict the uptake and transport of Cl- and Na+ ions from the rootstock to the above ground parts (Duran et al., 2003) [13]. Salinization of agricultural land is increasing and in many areas salinity management is critical for the successful crop production. In this view, identification of salt tolerant/resistant, high yielding and quality of fruits rootstocks of mango is important for future use. Hence, the present investigation was undertaken to understand the performance of monoembryonic and polyembryonic mango seedlings under salt stress condition.

Material and Methods
An experiment was conducted under net house condition (50 % light intensity) at Plant Physiology Laboratory, Division of Crop Production, Central Institute for Subtropical Horticulture, Rehmankhera, Lucknow. The treatments comprised with 5 (five) treatments viz., control as no salt (T₀), 15.8 g NaCl (T₁), 31.6 g NaCl (T₂), 37.487 g NaCl (T₃) and 74.97 g NaCl/10 kg pot soil (T₄) on monoembryonic and polyembryonic mango seedlings in complete randomized design. Ten plants were randomly selected from nursery for biochemical observations after the application of treatments to the tagged plants. Chlorophyll content of leaf (a, b and total) were estimated as suggested by Arnon (1949) and Proline content was estimated as per the method described by Bates et al. (1973) Starch concentration was measured after the leaf had been washed with ethanol. Starch was dispersed in alkaline solution and hydrolysed with amyloglucosidase (Novo Nordisk Bioindustries Ltd, Bagsvad, Denmark), then its concentration was determined by spectrophotometry using a glucose analysis kit (Boehringer Mannheim Corp./Diffchamb, Lyon, France). Sugars were extracted with 80% ethanol and were estimated by the method of Dey and Harborne (1990). Total carotenoids were estimated by the colorimetric method on the spectrophotometer (Model: Jasco V-670 UV-VIS-NIR spectrophotometer, Japan) as suggested by Roy (1973) [30]. Data were analyzed statistically and compared treatment means at the 5 % level of significance (Panse and Shukatme, 1985) [30].

Result and Discussion
The result of mono and polyembryonic seedlings under salt conditions were elucidated in the table 1 and 2 with regards to biochemical parameters viz. proline, chlorophyll, reducing sugar, total sugar and starch.

Proline (µg/mg FW)
In polyembryonic seedlings, proline content was found at higher level than monoembryonic seedlings. Proline content gradually increased (µg/mg FW) in treatments with increased intensity of salinity stress. Among the five salinity levels, highest proline content was recorded in T₄ treatment (0.041 & 0.033) in polyembryonic and monoembryonic rootstocks, respectively.

It has been extensively reported that proline content increases due to salinity in the citrus and different plants. (James et al. 2002; Roussos & Pontikis 2007) [21, 33]. Reports indicate the proline contents provides also rapid mechanism for maintaining turgor and affects the solubility of various proteins (Abraham et al., 2003) [1] and protects them against denaturation under saline condition (Jantaro et al., 2003; Tonon et al., 2004) [12, 45].

More proline accumulates under salt stress in both leaf and root tissue (Aziz et al.1999) and Putatively protects against the osmotic potential generated by salt (Watanabe et al. 2000, Chen et al. 2007) [46, 42]. The similar result observed by Hsu et al. 2003; Seki et al. (2007). Jiping Liu (1997) [20, 39, 23] in response to drought and salinity stress, many plant species accumulate high levels of proline, which is thought to function in stress adaptation.

Total chlorophyll
Total chlorophyll content was reduced in all treatments grown under salt stress conditions. Chlorophyll contents were decreased with increasing salinity level and maximum inhibiting effect was recorded in (T₄) in both the rootstocks. Maximum salinity level (T₄) resulted in chlorophyll minimum content of 1.23 µg/mg fw in poly and 0.87 µg/mg fw in monoembryonic rootstocks.

Similar finding were reported by Gu et al. 2004 that decrease in chlorophyll contents due to salinity. Most studies show that salinity adversely affects chlorophyll content (Meloni et al. 2003) [20].

As the result it is evident that chlorophyll contents in pea were decreased with the increasing salinity level and maximum inhibiting effect was recorded at high salt stress (75 mM) (Shahid et al., 2012) [9]. The lower chlorophyll content at higher salinity level also indicates stress and damaged the photosynthetic apparatus and decline the level of photosynthetic pigments may be attributed to salinity induced inhibition of chlorophyll biosynthesis (Khan, 2006 and Anastasia et al. 2013) that may be caused by the induced nutrients deficiency.

Carotenoids
Carotenoid concentration decreased with increasing NaCl concentration. Carotenoid level in the treatments was lower than the control value (0.179 & 0.237 mg/g fw, respectively), when the plants were grown under salinity stress and found lowest 0.14 and 0.193 mg/g in mono & polyembryonic rootstocks respectively. Similar findings were reported by Singh et al., 2008; Saikachout et al., 2013 and Sairam et al., 2002 have also suggested that carotenoids may be one of the required factor salt tolerances in crop plants and therefore, carotenoids content may be helpful to differentiate between salt sensitive and salt tolerance in plants.

Reducing sugars and total sugar
Salt intensity is increased in reducing sugar and total sugar in all the monoembryonic and polyembryonic seedlings. The maximum reducing sugar and total sugar concentration was recorded under higher salt intensity level as compared to control seedlings. Reducing sugar in both monoembryonic and polyembryonic seedling as accumulated (T₀ to T₄) were 0.003, 0.004, 0.005, 0.008, 0.014 and 0.003, 0.003, 0.0040, 0.006, 0.007, respectively. The lowest quantity of total sugar
was observed in control conditions in both monoembryonic and polyembryonic seedlings as compared to the treated ones. Total sugar concentration significantly increased with salinity stress.

The similar result has been reported by Balal et al. (2011) [10]. Salt stress caused an increase in total soluble sugars in all the citrus rootstocks. The maximum total soluble sugars concentration was recorded under higher salinity treatment (90 mM) as compared to control. More accumulation of total sugar is a common phenomenon under stress condition (William et al. 2000; Garg et al. 2002) [47, 17] reported an increase in total soluble sugar with progressive increase in salinity which have an important role in osmoregulation (Mohanty et al., 2002) [17].

A depletion of leaf carbohydrate concentration when citrus plants were grown under stress has been observed (Arbona et al. 2005) [7]. Another similar finding was observed (Perez et al. 2007) [32] a large reduction on leaf starch concentration in salinized plant.

### Table 1: Changing in biochemical molecule with salt regimes

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Proline (µg/mg Fw)</th>
<th>Total Chlorophyll (mg/g Fw)</th>
<th>Carotenoids(mg/g Fw)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Monoembryonic</td>
<td>Polyembryonic</td>
<td>Monoembryonic</td>
</tr>
<tr>
<td>T0</td>
<td>0.005</td>
<td>0.006</td>
<td>1.482</td>
</tr>
<tr>
<td>T1</td>
<td>0.008</td>
<td>0.011</td>
<td>1.384</td>
</tr>
<tr>
<td>T2</td>
<td>0.010</td>
<td>0.020</td>
<td>1.225</td>
</tr>
<tr>
<td>T3</td>
<td>0.015</td>
<td>0.025</td>
<td>1.113</td>
</tr>
<tr>
<td>T4</td>
<td>0.033</td>
<td>0.042</td>
<td>0.866</td>
</tr>
<tr>
<td>Mean</td>
<td>0.01</td>
<td>0.01</td>
<td>0.51</td>
</tr>
<tr>
<td>SEm+</td>
<td>0.000</td>
<td>0.000</td>
<td>0.005</td>
</tr>
<tr>
<td>CD at 5 %</td>
<td>0.001</td>
<td>0.001</td>
<td>0.016</td>
</tr>
<tr>
<td>CV</td>
<td>2.77</td>
<td>2.17</td>
<td>0.85</td>
</tr>
</tbody>
</table>

### Table 2: Changing in biochemical molecule with salt regimes

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Reducing sugar (%)</th>
<th>Total sugar (%)</th>
<th>Starch (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Monoembryonic</td>
<td>Polyembryonic</td>
<td>Monoembryonic</td>
</tr>
<tr>
<td>T0</td>
<td>0.003</td>
<td>0.003</td>
<td>0.393</td>
</tr>
<tr>
<td>T1</td>
<td>0.004</td>
<td>0.005</td>
<td>0.456</td>
</tr>
<tr>
<td>T2</td>
<td>0.006</td>
<td>0.005</td>
<td>0.464</td>
</tr>
<tr>
<td>T3</td>
<td>0.007</td>
<td>0.008</td>
<td>0.562</td>
</tr>
<tr>
<td>T4</td>
<td>0.010</td>
<td>0.014</td>
<td>0.635</td>
</tr>
<tr>
<td>Mean</td>
<td>0.002</td>
<td>0.003</td>
<td>0.210</td>
</tr>
<tr>
<td>SEm+</td>
<td>0.0002</td>
<td>0.0001</td>
<td>0.003</td>
</tr>
<tr>
<td>CD at 5 %</td>
<td>0.0005</td>
<td>0.0004</td>
<td>0.008</td>
</tr>
<tr>
<td>CV</td>
<td>5.03</td>
<td>3.96</td>
<td>1.02</td>
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</table>

### Conclusion

The current study indicates that increasing NaCl concentration exhibited inhibitory effect on total chlorophyll, carotenoid, reducing sugar, total sugar and starch except proline content in both monoembryonic and polyembryonic seedlings of mango. However, polyembryonic were found more tolerant to salt stress as compared to monoembryonic. Therefore, mango cultivar should be grafted with polyembryonic seedlings under such saline condition to grow successful cultivation mango.

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