Effect of Foliar Application of Salicylic Acid and Micronutrients on the Berries Quality of “Bez El Naka” Local Grape Cultivar

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ABSTRACT

This investigation aimed to study the effect of foliar application with some micronutrients alone or in combination with salicylic acid on physical and chemical properties of (Bez El Naka) grapevine cultivar during two successive seasons (2014 and 2015). Micronutrients (Fe, Zn, Mn and Br) were used as a mixture at a concentration of 50 and 100 mg/l in a chelated form in two different concentrations and salicylic acid at (100 and 150) mg/l. The result obtained proved that all parameters such as cluster weight, berries weight, juice volume, total chlorophyll content, N.P.K of leaves, T.S.S, acidity, total phenols and β-carotene were improved by all treatments as compared with control. Furthermore, treatments containing salicylic acid delayed ripening by about two weeks in comparison with the other treatments and the control.

Key words: Bez El Naka local grape cultivar, Salicylic acid, micronutrients, Total chlorophyll content, carotenoids, Total phenol.

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Introduction

Bez El Naka is one of the local grape varieties cultivated in Egypt especially in some of the old Delta Farms. However, limited area is cultivated with such variety in newly established farms that could be ascribed high postharvest berry losses and ability to lose water due to its thin skin and high rate of berry shatter. This cultivar is favored among local varieties due to its quality attributes such as cluster compactness, berry is oval and long, yellowish green at maturity, seeded with sweet taste and firm texture. It has many nutritive values as a major source of nutrients, organic acids, vitamins and many antioxidants such as total phenols and β-carotene. It has the advantage of midseason ripening time in early August.

Several epidemiological and clinical studies have reported the benefits of consuming fruits and vegetables high in polyphenols, resulting in reducing risks of developing cardiovascular diseases and several types of cancers (García-Alonso et al., 2004). Polyphenols are multifunctional, and their antioxidant activity may be due to their ability to act as reducing agents by donating hydrogen, capturing singlet oxygen, or acting as chelators (Bub et al., 2003). In generally, grapes are rich in phenolic compounds which have drawn attention not only for their important role in the development of grape products but also for their beneficial health effects (O’Byrne et al., 2002 and Fuleki and Ricardo-DA-Silva, 2003).

Phenolic compounds are considered one of the most important quality parameters for grapes and their products since they contribute to berry color and organoleptic characteristics such as flavor, bitterness, and astringency, in addition to the mentioned role as antioxidants (Gomez-Cordoves and Gonzalez-Sanjose, 1995).

Carotenoids are a widespread group of naturally occurring fat-soluble colorants, β-carotene which is the most abundant carotenoid in a human diet, its ability and degradation products undergoes single electron transfer-based reactions (SET). Carotenoids are used as a source for vitamin A and also related with a diminished risk for many degenerative disorders including various types of cancer, cardiovascular or ophthalmological diseases. The protective effects have been associated with their antioxidant activity, preventive cells and tissues from oxidative damage (Sies and Stahl, 1995, Mayne, 1996).

In grape berries the presence of carotenoids is well recognized. ß-Carotene and some xanthophylls (neoxanthin, flavoxanthin, and lutein) are abundant before veraison and subsequently decrease dramatically. Three other xanthophylls, namely, violaxanthin, luteoxanthin, and 5,6-epoxyxanthin, appear after veraison. Cultivar, viticultural region, exposure to sunlight, and ripening stage all affect carotenoid concentrations in grapes (Oliveira et al., 2004).

All plants need an adequate supply of macro- and micro-elements in order to match their normal physiological and biochemical function. Besides basic mineral nutrients (nitrogen, phosphorus and potassium), some other elements (magnesium, iron, zinc, boron, etc.) are considered to be essential for grapevine metabolism, growth processes, fruit development and quality because they are cofactors and/or activators of many metabolic enzymes (Ashley Rachel, 2011). The application of nutrients contributes to manipulate...
environmental variables when properly integrated into a soil management program. It can be used as a supplement to compensate for shortcomings of soils that is to provide adequate nutrients when nature does not supply them during the critical stages of the seasonal growth cycle (Keller, 2005).

Iron (Fe) is a cofactor for approximately 140 enzymes that catalyze unique biochemical reactions. Hence, iron has many essential roles in plant growth and development including chlorophyll synthesis, thylakoid synthesis and chloroplast development (Mousavi, 2011).

Zinc is one of the essential elements for plants. Zn is required for the synthesis of auxins, chlorophyll, starch and metabolism carbohydrate. Also, production of clusters with undeveloped shot berries and generally poor fruit set are all characteristics of Zn deficiency (Bybordi and Shabanov, 2010).

Manganese (Mn) is involved in photosynthesis and metabolism of nitrogen and carbohydrate (El-Sheikh et al., 2007).

Boron (Br) is responsible for activation of dehydrogenase enzymes, sugar translocation, nucleic acids and plant hormones. Addition to, is helpful in plant growth and productivity, fruit setting and yield in orchards. Also, is effect on cell wall structure cell elongation, root growth and transfer of sugar. Br deficiency has visual symptom on root and leaves growth, flower, cluster and berry development in grapevine. Severely reduced in internodes and shoot length, shoot tip death, low fruit set, and tiny berries are all common symptoms of boron deficiency. (El-Sheikh et al., 2007)

Salicylic acid (SA) is an endogenous plant growth regulator of phenolic nature and classified as a growth promoter, development and enhances plant vigour under biotic and abiotic stresses (Hayat et al., 2010). It is an important secondary metabolite in grape berries and plays an essential role in determining berry quality such as color, flavour, astringency and bitterness. SA prevents fruit softening by affecting activities of major cell wall degrading enzyme such as cellulase, polygalacturonase and xylanase (Srivastava and Dwivedi 2000, Zhang et al., 2003).

There are many soil and climatic factors affecting the availability of micronutrients to plants. The major factors include pH, soil water content, organic matter and interaction among the nutrients and temperature. In fact, foliar fertilization is applied in small droplets on the leaves and stems of the plant then nutrients are absorbed through these parts of plants. Foliar spray of micronutrients is advantageous over soil application, because of rapid response, effectiveness and elimination of deficiency symptoms due to certain micronutrients. Application of micro nutrients through foliage can be from 10 to 20 times as efficient as a soil application. (Kuepper, 2003 and El-Sheikh et al., 2007).

This research aimed to study the physical and chemical properties of this cultivar in response to micronutrients alone or in combined with salicylic acid to improve the transportation and marketing tolerance.

**Material and Methods**

This investigation was carried out during 2014 and 2015 seasons to study the effect of foliar application by some micronutrients alone or in combination with salicylic acid on physical and chemical properties of berries (Bez El Naka) local grape cultivar, grapevine cultivar. All grapevines were 10 year-old, spaced at 1.5x3 m. The vines were trained with double cordon system (leaving 16, 16 and 12 fruiting spurs x 3, 3 and 5 buds plus six replacement spurs x 2 buds, grown at the experimental vineyard, Pomology Department, Faculty of Agriculture, Assiut University. All vines received the standard agricultural practices that are used in the vineyard including soil fertilization, irrigation and pest control except for the tested different treatments through the two studied seasons. 35 vines were selected to be as uniform as possible.

Micronutrients (Fe, Zn, Mn and Br) were sprayed at a concentration of 50 and 100 mg/l in a chelated form in two different concentrations:-

1- Feed 1 {50 mg/L (Fe, Zn, Mn and Br) in a chelated form (Fe-EDTA, Mn-EDTA, Zn-EDTA and Borx)}.
2- Feed 2 {100 mg/L (Fe, Zn, Mn and Br) in a chelated form (Fe-EDTA, Mn-EDTA, Zn-EDTA and Borx)}.

Salicylic acid (SA) sprays in two different concentrations (100 and 150) mg/l.

All treatments were sprayed at the same day and twice a year, the first one was achieved at 12 May (after14 days from berry set) the second was carried out after 5 weeks of the first spraying. Micronutrients sprayed on leaves only but SA sprayed in both leaves and clusters. Triton B as a wetting agent at 0.1 % was added to all micronutrients and salicylic acid solutions. Sprays were applied in the morning (6-9 Am.) using a hand pressure sprayer.

The vines were sprayed with the following solutions:-

T1= Feed1
T2= Feed2
T3= Feed 1 + S.A100 g/l/vine.
T4= Feed1 + S.A150 g/l/vine.

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T5= Feed2 + S.A100 g/l/vine.
T6= Feed2 + S.A150 g/l/vine.
T7= Water spray (Control).
Each treatment sprayed 5 vines and replicated 3 times and was taken two clusters from each vine.

1-Leaf constituents
At harvest date, leaves were collected from mature leaves (5-7th leaves from shoot top) opposite to basal clusters to determine total chlorophyll content and N.P.K content.

Total chlorophyll content in the leaves:
Chlorophyll content was determined using a SPAD-502-meter (Minolta Camera Co., Osaka, Japan).

N.P.K Contents:
Leaf samples were washed first by tap water, then with distilled water and non-ionic detergent. Then they were dried with an air oven at 70°C and subsequently grind manually with mortar and pestle. One gram of the powder was burned in muffle oven at 550± 25°C. The resulting white ash was then dissolved in 10 ml of 2 N HCl and adjusted to a volume of 100 ml by distilled water for determining macro- and micro-nutrients (Chapman and Pratt, 1961). Potassium was determined by flame photometer and phosphorous content determined by spectrophotometer. Total nitrogen content was determined using Kjeldhal method (Olsen, et al., 1954 and Jakson, 1973).

2- Berries and juice properties:

Physical properties:
Two clusters were taken randomly from the yield of each vine 3 replicate and made as a composite sample for physical and chemical determinations.

*Cluster weight and weight of 50 berries/cluster were determined in grams by using analytical balance.
*Juice volume of 50 berries was determined in ml by Graduated cylinder.
*Chemical properties:-
*Total soluble solids in the juice were determined by using a hand refractometer.

Acidity in the juice was calculated as ml of tartaric acid per 100 ml of juice through titration against 0.1 normal sodium chloride using phenolphthalein as an indicator as outlined in the (A.O.A.C., 1985).

\[ \text{Acidity} \% = \frac{\text{Standard solution (N) x base solution (ml) x 0.075 \times 100}}{\text{Total juice (ml)}} \]

The equivalent weight of Tartaric acid = 0.075.
Total juice= 5 ml

TSS/Acid ratio: - \[ \frac{TSS}{\text{Total Acid contents}} \]

Carotenoids extraction and separation:
Total carotenoids were extracted from 20 g fresh grape using a mixture of methanol/ethyl acetate/petroleum ether (1:1:1, v/v/v). After filtering the extract, the residue was re-extracted twice with the same mixture of solvents, following the procedure described by (Breithaupt and Schwack 2000.) The extracts were combined and partitioned in a separation funnel, successively with water, diethyl ether and saturated saline solution. The ether phase was evaporated to dryness under vacuum, using a rotary evaporator at 35°C.

Total phenol:
The Folin–Ciocalteu method (Slinkard and Singleton, 1977) was used for the determination of the total phenol. In brief, an aliquot (1 ml) of the appropriate diluted extracts was added to a 10 ml volumetric flask, containing 5 ml of distilled water. Then, 0.5 ml of Folin-Ciocalteu reagent was added and the contents mixed. After 3 min, 1.5 ml Na2CO3 solution of concentration 5 g/L was added and made up to the total volume of 10 ml distilled water. After keeping the samples at 50°C (water bath) for 16 min in sealed flasks and subsequent cooling, their absorbencies were read at 765 nm against distilled water as the blank. A calibration curve was constructed using Gallic acid standard solutions (0–100 mg/L). The concentration of total phenol is expressed as the Gallic acid equivalent (GAE) per 1 g of fresh sample. All samples were prepared in triplicate.
Total phenol and β-carotene (mg/kg):

Total phenol and β-carotene (mg/kg) were determined by using Apparatus: Spectrophotometer double beam, Labomed, INC. Model: UVD-2950, Glass Cell.

Statistical analysis:

The experiment was arranged as Randomized complete blocks design with three replications. Analysis of variance (ANOVA) was carried out with SPSS software. Differences between means were assessed by Duncan’s multiple range tests with differences being considered significance at P < 5%.

Table of Analysis of the tested soil before starting the study

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<td>Silt (%)</td>
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<tr>
<td></td>
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Results and Discussion

1- Leaf constituents:-

Total chlorophyll content:

Result in Fig 1 demonstrated that, the total chlorophyll content was increased by SA and micronutrients spraying and there was a significant difference in total chlorophyll content associated with all treatments compared with the control. The highest values were noticed in leaves which were sprayed with T₁ and T₂ (52.5 and 51.1) then T₅ and T₆ were (49.3 and 50.6), respectively compared with the other treatments in the first season. Otherwise, foliar application with T₂ was significantly affect chlorophyll content than the other treatments and recorded (52.97) in the second season. These proved that treated with the high rate of micronutrients only or in combination with SA have been got the highest rate of chlorophyll content in the leaves.

Fig. 1: Effect of foliar application of salicylic acid and micronutrients on total chlorophyll content of leaves on Bez El Naka Grape during 2014 and 2015 seasons

*Means +SE expressed with bars with lines. (n=3) separation by Duncan's multiple range test at P <0.05. The same letters within Columns are not significantly different. Ascending order starts from (a or A) means the lowest value until reaches to the letter which has the highest value.

SA treatment increased leaf area, a subsequently rate of photosynthesis, total carbohydrates and photosynthetic pigments thereby, the bio-productivity of crops was increased (Hayat et al., 2010).
Mg is a main component of chlorophyll its contents in chlorophyll is about 15 to 20% of the total Mg constitution in plants. Also consider as a structural component in ribosome granules, stabilizing them in the composition necessary. Foliar spray of Mg increased the translocation of synthesized materials of the photosynthesis from the leaf to the grape fruit (Malakouti and Rezaei, 2001).

Iron forms are essential for both enzymes and chlorophyll synthesis. Accordingly, reducing iron in plants causes' leaf chlorosis, deficiency in vegetative growth, decreases net photosynthetic rate and chlorophyll content of plants. Zn and Fe spray application increased chlorophyll content of plants, leaf surface, net photosynthetic rate and vegetative growth (Pestana et al., 2001 and Álvarez et al., 2006).

N.P.K Content %:

Data concerning the effect of treatments on N, P and K % content during the two experimental seasons were listed in Fig 2 showed that, N and P contents were significantly increased in all treatments compared to the control. The highest value (2.44%) was found with sprayed T2 and T3 treatments and were more effective in N % content achievement than the other treatments in the first season and T2, T3 and T5 in the second season (2.5, 2.4 and 2.49%), respectively. The highest value of P % content was found in T2 treatment (0.55 and 0.56%), respectively and there was significantly increased in leaves compared with the other treatments in both seasons. The reduction of P % content in the other treatments might be explained due to that Zinc that decreases phosphorous content because there is a competitive effect between phosphorous and zinc in the uptake of ions. Thereby, treatments have a high rate of Zn show lower content of P % that will be achieved compared with the other treatments (Schreiner et al., 2006).

![Diagram showing the effect of foliar application of salicylic acid and micronutrients on N, P, K content (%) of leaves on Bez El Naka Grape during 2014 and 2015 seasons.](image)

**Fig. 2:** Effect of foliar application of salicylic acid and micronutrients on N, P, K content (%) of leaves on Bez El Naka Grape during 2014 and 2015 seasons

*Means +SE expressed with bars with lines. (n=3) separation by Duncan's multiple range test at P <0.05. The same letters within Columns are not significantly different. Ascending order starts from (a) means the lowest value until reaches to the letter which has the highest value.*

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There was a significant difference concerning K % content. The lowest values were detected with T2, T3 and T7 and there was an insignificant difference between them in the two seasons. On the other hand, the results showed the highest content associated with T4 in the first season and the other treatments (T1,T5,T6 and T8) on the second season (Martin et al., 2004).

On the other hand (Sarangthem and Singh 2003) proved that, the foliar application of SA enhanced the content of N, P, K, Cu, Fe, Zn, Na and Mn and increased the level of proteins and nitrate reductive activity.

2- Berries and juice properties:

Physical properties:

The results in Fig 3 demonstrated that, the physical properties were influenced by micronutrients and salicylic acid spraying. There were significant differences between control and the other treatments. The highest values were detected in cluster treatments and the lowest values were detected in the control (water sprayed) in two the investigated seasons.

Fig. 3: Effect of foliar application of salicylic acid and micronutrients on Cluster weight(g), 50 berries weight(g) and Juice volume on 50 berries(ml) on Bez El Naka Grape during 2014 and 2015 season.

*Means +SE expressed with bars with lines. (n=3) separation by Duncan’s multiple range test at P <0.05. The same letters within Columns are not significantly different. Ascending order starts from (a) means the lowest value until reaches to the letter which has the highest value.
Cluster weight, 50 berries weight and Juice volume of 50 berries:

During the two examining seasons, the results in Fig 3 showed that, the maximum cluster weight was in T6 (473.3g) in the first season and there was a significant difference between T6 and the other treatments. While in the second season, the treatments which contained different concentrations in both of micronutrients and SA had the highest cluster weight and no significant difference was detected between groups. There was a direct proportion between the concentration of SA and the cluster weight. It was observed that 50 berries weight and juice volume of 50 berries parameters achieved the same track of cluster weight. These results were found in harmony with the work of (Hayat et al., 2005 and Marzouk and Kassem 2011).

Foliar application of micronutrients (Fe, Zn, Mn and Br) had been associated with improved number of clusters, an average of cluster’s weight, juice content and the quality of vines. (Beede et al. 2005, Malakouti, 2007 and Akbar et al., 2013). Increasing in cluster weight which sprayed with B and Zn could be attributed to increase berry set, a number of berry in cluster and cell size or cell number resulting hence competition of photosynthetic substance between berries on a cluster (Ebadi et al., 2001). Increased berry volume was explained by increasing chlorophyll content in the leaf which is associated with high production of photosyhtate in a plant (Rana and Sharma., 1979).

Generally, to get the best price of table grapes in domestic and export markets, there are some characteristics for the cluster of grapes such as large berries, compactness cluster, firmness berries and sweetness. Foliar treatments with salicylic acid gave positive effects on these parameters might account for enhanced physical properties of cluster and berries. SA preserves berry firmness by affecting activities of prime cell wall degrading enzymes such as xylanase, cellulose, polygalacturonase and promotes cell division and cell enlargement (Hayat et al., 2005).

Chemical properties (T.S.S, Acidity, T.S.S/acid ratio, Total phenol and β-carotene):

T.S.S:

The obtained results of both seasons Fig 4 revealed that, the highest concentration of TSS was detected with T1 and T2 treatments compared with the other treatments.

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**Fig. 4:** Effect of foliar application of salicylic acid and micronutrients on T.S.S (°Brix), Total phenol (mg/kg) and β-carotene (mg/kg) on Bez El Naka Grape during 2014 and 2015 seasons

*Means +SE expressed with bars with lines. (n=3) separation by Duncan’s multiple range test at P <0.05. The same letters within Columns are not significantly different. Ascending order starts from (a) means the lowest value until reaches to the letter which has the highest value.*
In the first season, there was no significant difference between them and T7 control. In the second season, T1 and T2 had significant differences compared with the other treatments. Treatments which contain salicylic acid had fewer values of TSS may be due to the basic role of SA is delaying ripening more than other treatment. However, (Tareen et al. 2012) found that, the fruits which treated with salicylic acid (SA) showed reduced in TSS rate. Comparison between T3, T4 and T5, T6 proved that, TSS value in treatment which contained Feed 2 was higher than that contained Feed 1 despite that contained SA. This probably caused by using a high rate of micronutrients in both studied seasons.

**Acidity:**

Results in Fig5 demonstrated that, the lowest values of acidity were detected by T1, T2 and T7 treatments and the highest values were found in T3 and T4 (0.703,0.697) and (0.713,0.694), respectively compared to the other treatments in both seasons but no significant difference was detected. Acidity values that increased by the other treatments which contained SA might account to the function of SA which delayed ripening. Compared results between micronutrients treatments and treatments which included of SA proved that, the treatments which contained a higher amount of micronutrients, achieved lower acidity. (Moustafa et al., 1986,Elena et al., 1998)

![Fig. 5: Effect of foliar application of salicylic acid and micronutrients on Total acidity% on Bez El Naka Grape during 2014 and 2015 seasons](image)

*Means +SE expressed with bars with lines. (n=3) separation by Duncan’s multiple range test at P <0.05. The same letters within Columns are not significantly different. Ascending order starts from (a or A) means the lowest value until reaches to the letter which has the highest value.

**TSS/acid ratio:**

Data in Fig 6 indicated that, the clusters which treated with salicylic acid showed a delay in ripening compared with the other treatments. Higher rate of TSS/acid ratio in both studied seasons was insignificantly associated with T1, T2 and T7, respectively and the lowest value was found in T4 (20.8 and 21.59), respectively in both seasons.

It is known that the micronutrients have an important role in photosynthesis and enzymes responsible for plant metabolism so the greatest benefit effects of spraying micronutrients are improving the leaf area, growth, productivity and increasing total soluble solids and total sugars while reduced the total acidity of grapevines cvs (Singh, 2002).

Our results are in agreement with those of (Moustafa et al., 1986, Sourour, 2000, Malakouti and Rezaei, 2001) who reported that treated with Fe significantly increased TSS, sugar and decreased acidity. Fe plays a key role in carbohydrate metabolism and fruit quality. Application of Mg for grapes usually increases the sugar content supposed that a foliar application of Zn increased TSS. Zn has also an important role in photosynthesis and related enzymes which are resulted in decreasing acidity and increasing the sugar. A favorable effect of foliar application of Br might be due to its role in sugar metabolism and accumulation of carbohydrates.
Fig. 6: Effect of foliar application of salicylic acid and micronutrients on T.S/S/ acid ratio on Bez El Naka Grape during 2014 and 2015 seasons
*Means +SE expressed with bars with lines. (n=3) separation by Duncan's multiple range test at P <0.05. The same letters within Columns are not significantly different. Ascending order starts from (a or A) means the lowest value until reaches to the letter which has the highest value.

Total phenol:
Concerning data in Fig 4 the total phenol in both investigation seasons increased with all treatments compared with the control. Values of T2 was (66.25 and 67.16 mg/kg) associated with the highest concentration compared with the others and followed by T1 (59.19 and 59.49 mg/kg), respectively. There was a significant difference between all treatments compared with the control in two investigated seasons.

Compared results between both applications that included salicylic acid demonstrated that, usage of a high rate of micronutrients was more effective than the lower rate. Nevertheless, high concentration of SA reduced total phenol more than low one. So results proved that, the micronutrients improved quality of fruits including total phenol otherwise, SA increased total phenol content too but the main function of SA is delaying ripening more.

SA is as an elicitor of phenolics and hydrolytic enzymes involved in grapevine defense (Renault et al. 1996). Salicylic acid is formed from the phenylpropanoid metabolism via decarboxylation of E-cinnamic acid to benzoic acid and its subsequent hydroxylation. It is common to participate in plant defense mechanisms, especially in the systemic acquired resistance (SAR) (Ryals et al. 1996).

β-carotene:
The results in Fig 4 presented that, all treatments showed the significant values compared with the control. The highest concentration was recorded in T2 and T1 then T3 and T5, respectively and the other treatments came after them in both seasons. Data proved that, β-carotene was increased when the rate of micronutrients increased alone or combination with SA (Abdel et al., 2000).

(Moharekar et al., 2003) described that, salicylic acid stimulate the synthesis of carotenoids and xanthophylls.

Veraison is a major stage and a nature signal that initiates of grape berry developing and ripening. ABA (abscisic acid) hormone has the main role of berry ripening and its concentration increased during berry ripening. It is known that, there is an antagonistic direction between function of SA and ABA. SA application for grapevine berries delayed or inhibited ripening when applied at veraison stage. Researchers demonstrated that, the increase in ABA level, which started at veraison, may causes the ripening of grape berry. Therefore, SA could inhibit the effect of ABA (During et al. 1978, Ray 1986 and Raskin 1992).

Some researches proved that salicylic acid enhanced membrane permeability would that, facilitate absorption and utilization of mineral nutrients and transport of assimilates. This would also participate towards increasing the capacity of the treated plants for biomass production as it reflected in increasing fresh and dry weight of plants. Therefore, the application of salicylic acid had increased total soluble sugar (Chandra et al., 2007).

Boron is investigated to be developing the phloem carbohydrate and sugar movement which may increase fruit soluble solid content. Thus, TSS can accumulate very rapidly with use suitable nutrition like boron thus
enhancing translocation of sugars from leaves to the fruit which can be postulated that it hastens maturity. (Hanson, 1991 and Jonathan, 2012)

References


