Effect of salt stress on physiological response of tomato fruit grown in hydroponic culture system

M.M. Hossain[1], H. Nonami[1,2]

[1]*The United Graduate School of Agricultural Sciences, Ehime University, Matsuyama, Japan*
[2]*Plant Biophysics/Biochemistry Research Laboratory, Faculty of Agriculture, Ehime University, Matsuyama, Japan*

Abstract

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The effect of salt stress on physiological response of hydroponically grown tomato fruit was investigated. Fruit growth rate, water status, cuticle permeability and induction of blossom-end rot (BER) of tomato fruit were considered for this study. Salt stress was applied by using Ca salt treatment and it plays an important role on all parameters studied in this experiment. Fruit growth rate, predawn water potential, osmotic potential and cuticle permeability were significantly lower in treated plants than in control plants. On the other hand, tissue turgor of control and treated fruit showed almost similar values 12 days after flowering (DAF). This result indicated that turgor was osmotically regulated in fruit under stress condition. Fruit growth rate was found to decline from 12 DAF and eventually ceased when BER externally appeared on fruit surface at the age of 19 DAF in this experiment. The reduction of growth rate coincided with the reduction of water potential in fruit tissue due to salt stress. Although BER externally appeared at 19 DAF anatomical investigation showed that intercellular air space becomes discoloured at least one week before external symptoms appeared on fruit tip. Different levels of cuticular permeability indicated that the deposition of cuticular wax on fruit surface was enhanced by the salt stress condition in tomato fruit. Since, BER was found to appear on fruit tip under high calcium concentration in solution it can be concluded that calcium deficiency was not the only the cause of BER in tomato, rather salt stress might alter metabolic activity in developing tomato fruit.

Keywords: cuticle permeability; fruit expansion; physiological disorder; water status

Tomato fruits suffer from various physiological problems in hydroponic culture as well as soil culture around the world. Until now the causes behind such kind of abnormalities in tomato cultivation have not been well understood. Blossom-end rot (BER) is one of the physiological disorders in tomato fruit which causes severe production loss. Wada et al. (1996) reported that calcium concentration of young fruit decreased quickly when fruit fresh weight reaches an average of 20 g and BER began to appear on fruit tip. The occurrence of BER in tomato was reported in many scientific literatures and most of them identified that local calcium deficiency in the fruit tip is the main cause of BER in tomato (Bradfield, Guttridge 1984; Adams, Ho 1992, 1993; Ho, White 2005). It is possible that local Ca deficiency for individual cells in the distal tissues might be responsible for BER in tomato (Schmitz-Eiberger et al. 2002; Suzuki et al. 2003). Liebisch et al. (2009) found that calcium spray on tomato fruit decreased the incidence of blossom-end rot. It is widely reported that local deficiency of calcium plays the key role for the induction of BER in tomato fruit. Nonami et al. (1995) postulated that calcium concentration in normal and BER infected fruits was similar and concluded that calcium deficiency in fruit may not be the direct cause of occur-

rence of blossom-end rot in tomato. BER in tomato can also be severe when tomato is exposed to high vapor pressure (Bertin et al. 2000). Some researchers described that when transpiration demand is high, a higher amount of calcium is absorbed by leaves as compared to fruit (Ho 1989; Adams, Ho 1992, 1993). However, calcium is necessary for cell wall synthesis, enzymatic activity, metabolism and maintaining the integrity of cell wall during rapid expansion of fruit in the early stage of fruit development. Calcium is associated with the middle lamella of cell walls playing a role in support and growth of cell (Wu et al. 2002). It is deposited in plant cell wall during cell wall synthesis. It is necessary for the stability of cell membrane and works as a cementing agent in the cell wall as calcium pectate and binds the cells together so that any shortage and/or excess of calcium during rapid cell expansion may cause metabolic disorder during fruit growth. There are several possibilities for the low level of calcium in the fruit tip such as supply of low calcium content in nutrient solution; translocation of calcium to the fruit tip is restricted by high levels of GAs during rapid fruit growth period (Saure 2005). Calcium is a slowly moving element and it is passively transported by the xylem through the transpiration streams from leaves and fruits. It is reported that tomato fruit surface has no stomata thus cuticular transpiration is the only way of water movement from fruit to atmosphere. It is assumed that translocation of calcium is low in fruits especially in the fruit tip due to low cuticular transpiration in the fruit tip. Fruit cuticle could be the factor causing BER in tomato. The cuticle plays an important role in inhibiting transpiration from plant surface (Vogg et al. 2004) which consists of wax, cutin and phenylpropanoids. The deposition of epicuticular wax on fruit surface may have some relation with the concentration of Ca^{2+} in cell wall which may cause BER in fruit while excess Ca^{2+} present in cell wall. The objective of this study was to explore the effect of calcium salt stress on growth, water status, and cuticle permeability of tomato fruit as well as on induction of BER in tomato fruit.

MATERIAL AND METHODS

Plant materials

Tomato seeds (*Solanum lycopersicum* L. cv. Momotaro) were germinated in the laboratory at 25 ± 1°C in the dark in February 2010. Two-week old seedlings were grown hydroponically in plastic planter using Otsuka-house nutrient solution No. 1 & 2 (Otsuka Chemical Co., Ltd., Osaka, Japan). Excess calcium ($CaCl_2 \cdot 2H_2O$) was added into above nutrient solution and elevated solution electrical conductivity (EC) 8 mS/cm which provided additional 32.3mM Ca/l into regular nutrient solution. Before application of treatment, all plants were grown with the same concentration of solution (EC 1.0 mS/cm) then solution with EC 8 mS/cm was applied when flowers fully bloomed in the first truss. At the same time EC 1.5 mS/cm was used for control plants. Flowers were artificially pollinated by using hormone and days after flowering (DAF) was considered from the date of hormone application.

Measurement of fruit growth and hydraulic permeability of fruit cuticle

The vertical expansion of fruit was measured with an extensometer (Nonami, Boyer 1990). A selected fruit was fixed with a horizontal rigid reference metal bar attached to the microscope stand with the help of a cloth adhesive tape so that fruit could be connected easily to the transformer. After that the head of a T-shaped tiny metal pin was connected at tip of fruit with cloth adhesive tape and the tail of pin was cramped with aluminum bar attached to the rotary variable differential transformer. Fruit expansion was continuously recorded with a recorder (OMNIACE II, RA 1200; NEC San-ei Instrument, Ltd., Tokyo, Japan). Growth rates of fruit were calculated from the slope per unit time of the length displacement. Water movement through fruit cuticle was determined from intact tomato fruit by using a pressure chamber technique. A certain magnitude of pressure was applied to create water potential gradient from outside to inside of fruit cuticle. A defined fruit surface area at fruit tip was kept under distilled water to allow water entry through fruit cuticle. Compressed air was applied to the pressure chamber slowly and water was moved into fruit cuticle due to water potential gradient; after fluid was collected from pedicel cut surface with a micro syringe for a certain time period. The flow of water can be determined from the following equation:

$$F = \frac{v}{t} \text{ (µl/s)}$$

where:
F – flow rate
v – volume of fluid (µl)
t – time (s)

The water movement across the fruit cuticle can be described by Fick's law of diffusion according to the following equation:

$$\frac{F}{A} = J = D \times \Delta P, \quad D = \frac{J}{\Delta P} \qquad (1)$$

where:

A – unit time divided by fruit surface area (cm^2)

J – water flux (µl/cm^2/s)

D – conductance of cuticle (µl/cm^2/s/MPa)

ΔP – pressure gradient (MPa)

Measurement of water status with isopiestic thermocouple psychrometer

Water potential (ψ_w), osmotic potential (ψ_s) and tissue turgor (ψ_p) of control and treated fruits were determined by using isopiestic thermocouple psychrometer according to the methods described by Nonami and Boyer (1987); Boyer (1995). At 12 DAF fruits were used for water status measurement from both treatments. Fruit samples were collected from the greenhouse at predawn (i.e., between 5 to 6 a.m.) and following all consecutive operations were conducted at saturated condition in a globe box. About 1 cm^2 size fruit tissue was collected from the fruit tip from where BER symptom supposed to appear. At the same time water potential of nutrient solution in the pot was determined by using similar technique.

Determination of BER incidence in fruit

Tomato fruits of treated and control plants were visually inspected daily to find out the time of BER symptoms. Moreover, it was also examined in cellular level under microscope. A free hand cross section was made from fruit tip pericarp tissue and washed out several times with distilled water; then it was observed under microscope.

RESULTS

Effect of salt stress on fruit growth rate

During the experimental period, the three dimensional fruit volume was directly measured from intact plants in the greenhouse; after that growth rate was calculated as volume change as a function of time (day). The result showed that there was no significant difference of fruit volume and growth rate between control and treated fruits from 6 to 12 DAF (Fig. 1a, b). In treated fruits both parameters were found to decline significantly from 14 DAF. A brown colour necrotic area was found on pericarp in the distal end of fruit after 19 DAF (average). The vertical expansion rate of intact fruit was also determined by using rotary variable differential transformer from control and treated plant. In this case, 12 DAF fruit was used for measuring vertical expansion of fruit. The length displacement and growth rate were found significantly different between control and treated fruits (Fig. 2a, b). The average growth rate of fruit of control plants was 0.0264 (×10^{-6} m/s) while fruit growth rate of treated plants was 0.013 (×10^{-6} m/s) (Fig. 2c). Fruit growth rate was also measured from control fruits at 10, 20, 30 and 40 DAF. The results showed that

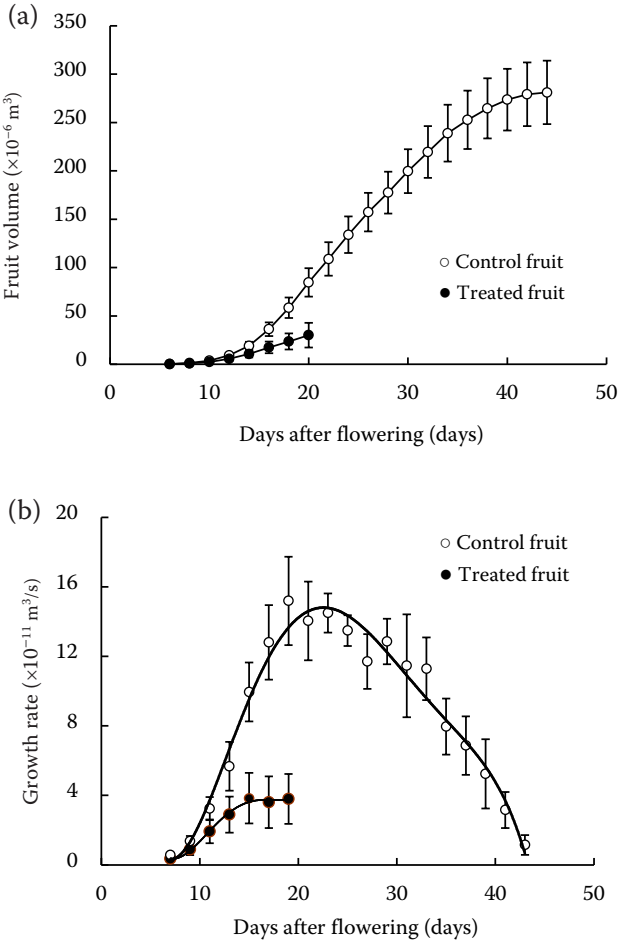

Fig. 1. Volume (a) and growth rate (b) of control and treated fruits were measured from the same fruits in the greenhouse during entire growth period. In case of treated fruits, BER symptoms appeared almost after 19 days after flowering (average). Bars indicate 95% confidence intervals calculated from the Student's *t*-distribution

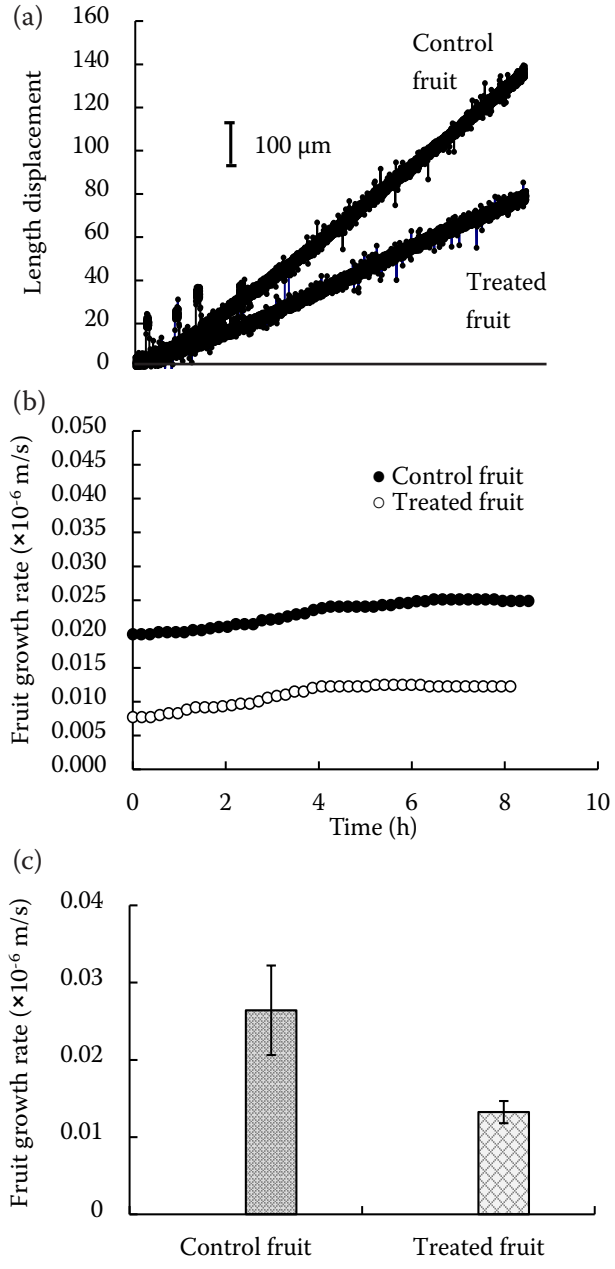

Fig. 2. Length displacement (a), growth rate of individual fruit (b) and average growth rate (c) of control and treated fruits at 12 days after flowering. Bars in C indicate 95% confidence intervals calculated from the Student's *t*-distribution

the growth rate of fruits declined significantly with increasing fruit age (Fig. 5 a,b).

Effect of salt stress on water status of fruit

Predawn water status was determined from control and treated fruits by using isopiestic thermocouple psychrometer. Tissue water potential (ψ_w) and osmotic potential (ψ_s) were significantly higher in control fruits than in salt stressed fruits at 12 DAF (Fig. 3a). The average water potential and osmotic potential of control fruits were –0.58 MPa and –0.88 MPa, respectively. In treated fruits, the average values of water and osmotic potentials were –0.79 MPa and –1.04 MPa, respectively (Fig. 3a). Although the values of fruit tissue turgor (ψ_p) were slightly higher in control fruits (0.29 MPa) than in treated fruits (0.24 MPa) the difference was not significant between two treatments (Fig. 3a). The water status was also determined from pot solu-

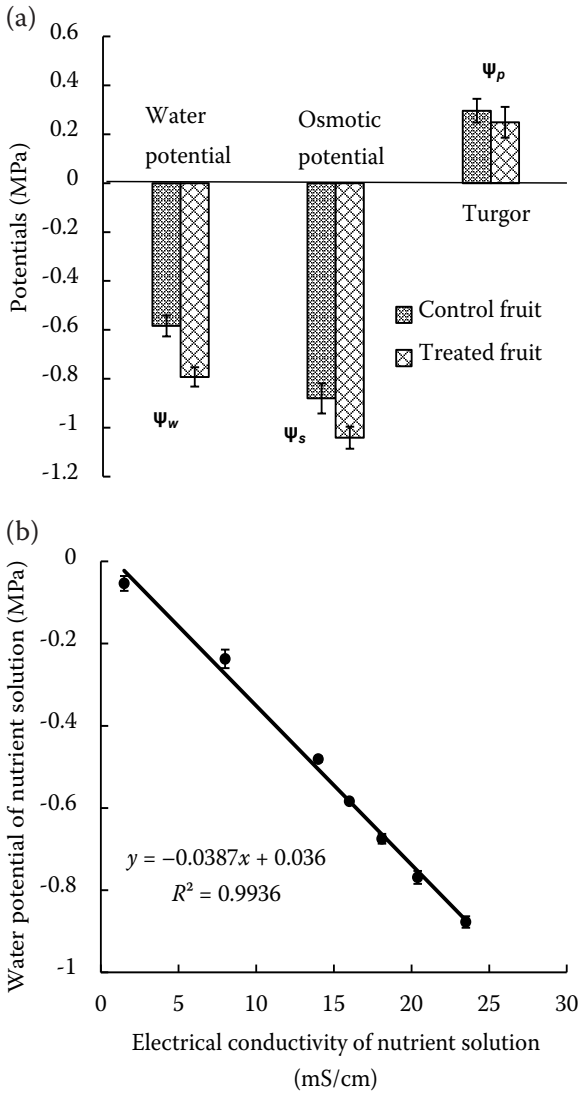

Fig. 3. Water potential (ψ_w), osmotic potential (ψ_s) and turgor (ψ_p) of control and treated fruits at 12 days after flowering (a) and water potential of nutrient solution in pots at various electrical conductivity (EC) during entire growth period of tomato plants in the glasshouse (b) were measured using psychrometers. Bars indicate 95% confidence intervals calculated from the Student's *t*-distribution

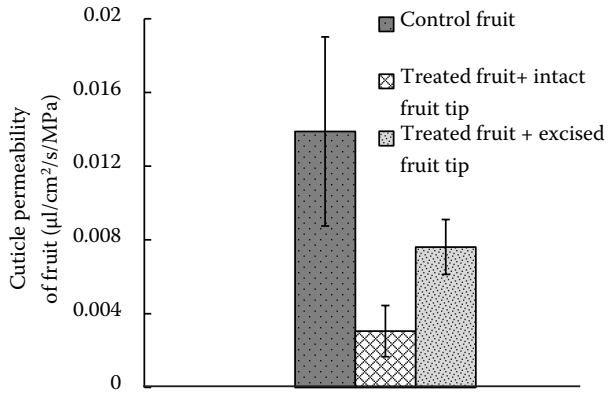

Fig. 4. Water permeability of fruit cuticle of control and treated fruits were measured at 12 days after flowering. Cuticle permeability was measured from fruit tip using pressure chamber technique. Bars indicate 95% confidence intervals calculated from the Student's *t*-distribution

tion and the result showed that the electrical conductivity and water potential of pot solution were correlated (Fig. 3b). Water potential of solution was found to decrease with increasing electrical conductivity of solution due to water uptake by plants.

Effect of salt stress on fruit cuticle permeability

Water permeability of fruit cuticle of intact tomato fruit was determined according to Eq. (1). The average cuticle permeability of control fruits was found significantly higher than that of treated fruits with intact fruit tip (Fig. 4). The values of cuticle permeability of control and treated fruits with intact fruit tip were 0.013 and 0.003 µl/cm^2/s/MPa, respectively. The permeability of cuticle of treated fruits was elevated after excision of fruit tip immediately after measurement with intact fruit tip. There was a significant difference between "before" and "after" excision of fruit tip of treated fruits (Fig. 4). This result indicated that cuticle was the main barrier for water movement from outside to inside of fruit. Cuticle permeability was also measured from control fruits at 10, 20, 30 and 40 DAF. The results showed that the cuticle permeability declined significantly with increasing fruit age (Fig. 5c).

Effect of salt stress on BER incidence

Fruits of treated and control plants were inspected daily to identify the time of BER symptom that appeared externally on fruit tip tissue. BER in tomato fruits of treated plants became visible externally after 19 DAF. There was no such BER symptom found in control plants during the experimental period. From the anatomical view of treated fruit tip tissue at 12 DAF, it was clearly observed that a slightly black discolouration appeared in the intercellular air space and also in the cell wall. The discolouration became

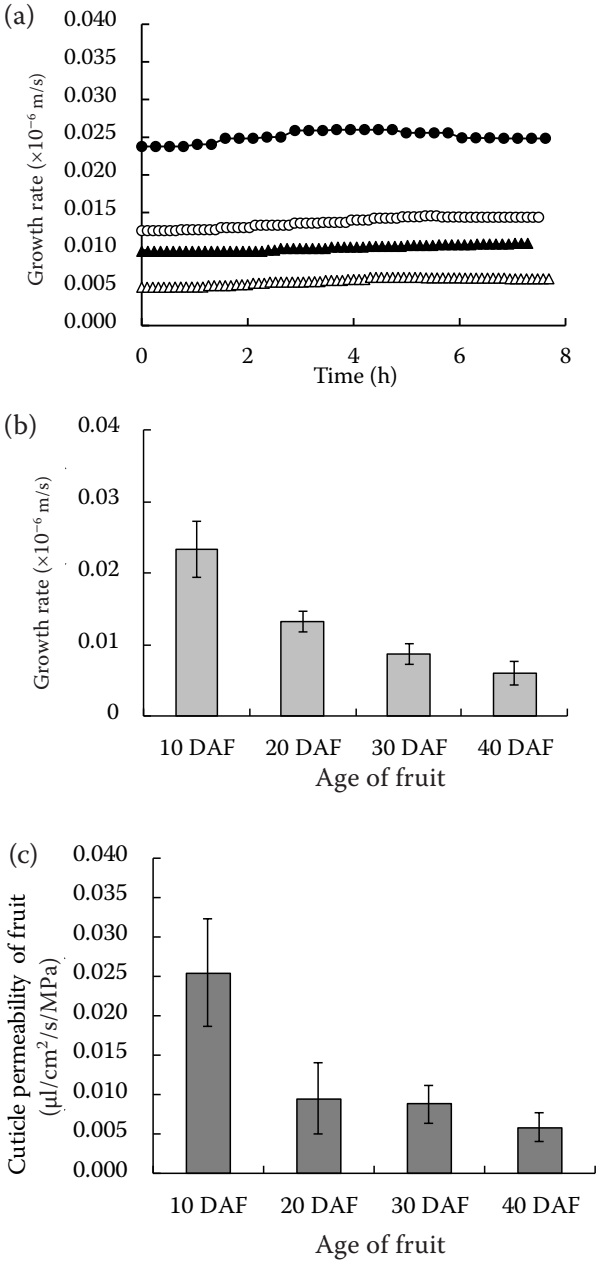

Fig. 5. Individual growth rate (a), average growth rate (b) and cuticle permeability (c) of tomato fruit. In (a), symbols are 10 days after flowering (DAF) (●), 20 DAF (○), 30 DAF (▲) and 40 DAF (Δ). In (b) and (c), bars indicate 95% confidence intervals calculated from the Student's *t*-distribution

darker and extended more area of cell wall within a week. From this observation, it was indicated that although BER appeared externally after 19 DAF it was induced at the cellular level at 12 DAF.

DISCUSSION

In this study, we demonstrated the effects of salt stress on physiological response of tomato fruits grown hydroponically in the greenhouse. Fruit growth rate, water potential, osmotic potential and cuticle permeability decreased significantly in treated fruit as compared to control fruit. BER in tomato fruit became visible externally at the age of 19 DAF but internally black discolouration was found in the intercellular air space at least one week before i.e., at 12 DAF. During this period, fruit growth rate was rapid and maximum growth rate was observed at 19 DAF in control fruits (Fig. 1b). Growth rate of treated fruits were found to decline significantly after 14 DAF and it almost ended after 19 DAF. It was reported that initial rapid growth rates play an important role in the induction of BER in tomato (Ehret, Ho 1986). They reported that the induction of BER in tomato fruit was related to low calcium concentration during rapid fruit growth period. In this experiment, 3.52mM Ca/l was present in nutrient solution for the control treatment while treated plant experienced over 10 times higher Ca concentration in solution i.e., 35.83mM/l, and plants were grown in the same environmental condition in a greenhouse. Under such a condition treated plants produced BER symptom on fruit tip but control plants did not have such symptoms. It was assumed that an over-dose of Ca ion concentration might have caused the induction of BER in treated fruit. In the earlier studies a similar concentration of Ca ion was found in fruits having BER and control fruits (Nonami et al. 1995). They suggested that Ca deficiency in fruits may not be the direct cause of BER in tomato. As a whole, salt stress may reduce water uptake ability of plant and ultimately reduce growth rate as well as change metabolic activity (Munns 2002). They stated that if excess amount of salt entered into plant, this salt finally rises to toxic level in leaf tissue which can cause early senescence of leaf. Finally, it reduces the photosynthetic capability of plant and retards the growth rate of plant and its other organs. In this experiment, growth rate of treated fruit was significantly declined compared to control fruit (Fig. 2c). This result is in agreement with the statement of Munns (2002). There was a positive correlation between solution water potential and electrical conductivity. As water was absorbed by plants during growth and development and electrical conductivity of pot solution was gradually increased and water potential became more negative. In such conditions water potential of treated fruits became lower than that of control fruits measured from fruit tip tissue. As water potential of treated fruits was declined, osmotic potential showed similar trend and had lower osmotic potential in treated fruits than in control fruits; the turgor pressure in tissue level showed a non-significant difference between two treatments. It indicated that osmoregulation in tissue level maintains turgor as high as control fruit.

A significant progress was made during the last couple of years in analysing and understanding transport mechanisms of cuticular membranes of plants (Petracek, Bukovac 1995; Kerstiens 1996; Knoche et al. 2001). In most cases the analyses of cuticular membrane permeability was based on excised tissue of plants. In this experiment, intact tomato fruit was used to determine cuticle permeability of fruit with a pressure chamber technique. Water permeability of tomato fruit cuticle was markedly changed during fruit development (Fig. 5c). There was a negative correlation between cuticle permeability and fruit age. At 10 DAF cuticle permeability was significantly larger than at 20 DAF and more mature fruits (Fig. 5c). The decline of permeability with increased fruit age might have caused deposition of cuticular wax on fruit cuticle surface. Knoche et al. (2000) showed that wax represents a primary barrier to water penetration in the sweet cherry fruit cuticular membrane. In this experiment, we applied salt stress treatment and cuticle permeability was measured at 12 DAF both from control and treated fruits. The cuticle permeability of treated fruits was significantly lower than that of control fruits and it increased after excising the fruit tip of treated fruit. This result corroborated with the result of Vogg et al. (2004). They stated that cuticular permeability of tomato fruit was increased when epicuticular wax was removed. The permeability of treated fruit at 12 DAF was similar with the permeability of control fruit at 40 DAF. This results indicate that cutinization of epidermal cell wall of treated fruit became rapid due to salt stress.

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Corresponding author:

Assoc. Prof. Dr. Md. Mokter Hossain, Bangladesh Agricultural University, Faculty of Agriculture, Department of Horticulture, Mymensingh 2202, Bangladesh
phone: + 880 1748 020966, fax: + 880 916 1510, e-mail: hossainmdmokter@gmail.com