Effects of Different Potassium Nitrate Concentrations and Temperature Conditions on the Elemental Composition and Blossom-End Rot in Paprika (Capsicum annuum L.)

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Blossom-end rot (BER) in paprika (Capsicum annuum L.), as in tomato fruits, is a physiological disorder caused by calcium (Ca) deficiency in fruits. While studies have revealed that excessive nitrogen and potassium fertilization and high temperatures affect Ca transport to fruits and induce BER, few studies have investigated the effects of other elemental concentrations in paprika fruit and their association with BER occurrence. This study aimed to investigate the relationship between the changes in the elemental composition and incidence of BER in paprika fruits grown at different potassium nitrate (KNO₃) concentrations and different temperature conditions. Paprika plants were grown in rockwool blocks in a greenhouse at 25°C and 30°C for approximately four months and irrigated with liquid fertilizer and three different concentrations (0, 27.9, and 74.4 mM) of KNO₃ were added. Subsequently, the total fresh weights of ripe paprika fruits and the incidence of BER were measured, and the elemental compositions in the pericarp of the first and last sampled fruits were analyzed. The total fresh weights significantly decreased and the incidence of BER increased with increasing KNO₃ concentrations and temperature. The profiles of 11 mineral elements in the pericarps of paprika fruits revealed a significant positive or negative correlation between not only the concentration of Ca, but also that of several other elements including boron (which crosslinks pectin in the cell wall like Ca) and the incidence of BER. These results suggest that Ca deficiency may not be the only cause of BER occurrence, and that several elements may also be involved. The insights from this study will contribute to help predict the incidence of BER and stabilize crop production by improving fertilizer application and environmental control.

Key Words: boron, calcium, fruit pericarp, ionome, pectin.

Introduction
Paprika (Capsicum annuum L.) plants are important vegetable crops that are grown mainly in greenhouses worldwide. Inappropriate cultivation of paprika plants in plant factories, for example, under excessive application of fertilizers or long-term high temperatures, can lead to a variety of physiological disorders and a reduction in the marketable yield of the fruit. Blossom-end rot (BER) is a serious physiological disorder in which brown necrotic tissue expands from the distal part of the fruit (Taylor and Locascio, 2004). In pepper, the calcium (Ca) concentration decreases from the proximal to the distal end of the fruit, where BER occurs, and a similar observation has been reported in tomatoes (Adams and Ho, 1992). Although Ca deficiency is generally considered as the primary cause of BER, a combination of other factors that disturb the supply of Ca to the fruit also play a role in BER occurrence.

The incidence of BER has also been shown to be
associated with excessive application of nitrogen (N) and potassium (K) fertilizers (Ho et al., 1993; Bar-Tal and Pressman, 1996; Taylor and Locascio, 2004). High levels of N, which promote plant growth, have been suggested to increase the rate of transpiration and thus promote the movement of Ca towards the leaves, reducing the Ca movement towards the fruits (Ho and White, 2005). High levels of K have also been shown to induce Ca deficiency in fruits (De Freitas et al., 2018) and increase the incidence of BER (Bar-Tal and Pressman, 1996). In addition, high levels of both N and K are known to be involved in cell expansion, which may promote rapid fruit growth and lead to Ca deficiency (Bar-Tal et al., 2001; Elumalai et al., 2002; Indeche et al., 2020).

The effect of high temperature on fruit Ca concentrations has also been demonstrated. High temperature conditions have been considered to accelerate fruit expansion, as well as increase N and K levels, and rapid fruit expansion may cause a temporary decrease in Ca concentration, resulting in BER incidence (De Kock et al., 1982; Ho and White, 2005). In addition, increasing temperatures can potentially reduce relative humidity (RH), which has been shown to enhance fruit susceptibility to BER (Adams and Ho, 1993).

Although many studies have investigated the effects of high levels of N and K and high temperature on Ca transport in fruits, few studies have investigated the effect of the concentrations and transport of other elements in fruits and their association with BER incidence. It has been reported that the overexpression of the genes involved in Ca transport in fruits results in an increased probability of BER occurrence regardless of the increase in fruit Ca accumulation (Park et al., 2005), suggesting that deficiencies in essential elements in fruit other than Ca may lead directly or indirectly to BER incidence. Thus, this study aimed to investigate the changes in the elemental composition in paprika fruits grown at different potassium nitrate (KNO₃) concentrations and different temperature conditions; these changes are important to elucidate the detailed mechanism of BER and applying this knowledge to develop BER reduction technology.

Materials and Methods

Plant materials and growth conditions

Seeds of ‘ARTEGA’ (Enza Zaden, The Netherlands) paprika (Capsicum annum L.) were sown in cell trays on 27th April 2018. After germination, the plants were transplanted into 10 × 10 × 10 cm rockwool blocks (Grodan, The Netherlands) and irrigated with liquid fertilizer (Otsuka-A solution; OAT Agrio, Tokyo, Japan). On 14th June 2018, the plants were transplanted and cultivated under natural light conditions at Hokkaido University in a closed climate chamber set to a control regime (25/25°C day/night) or a high temperature regime (30/25°C day/night). The average daily solar radiation during the planting period was 16.9 MJ·m⁻².

For both regimes, the plants in rockwool blocks were placed on rockwool slabs (100 × 20 × 7.5 cm) at a density of 4 plants per slab and irrigated with Otsuka-A solution containing N (18.6 mM), P (1.7 mM), K (8.6 mM), Mg (1.5 mM), Ca (4.1 mM), Fe (48.4 μM), Mn (21.1 μM), Zn (1.4 μM), Cu (0.47 μM), boron (B) (21.6 μM), and molybdenum (Mo) (0.31 μM) to which KNO₃ was added at three different concentrations (0, 27.9 and 74.4 mM). The plants were trimmed to three stems of even length with one leaf and one flower per node.

The incidence of BER in all fruits was recorded, ripe BER and non-BER paprika fruits were sampled and weighted weekly from 20th August to 11th October, and the total weight was calculated for each plant. For mineral analysis, non-BER fruit and leaf samples closest to the fruit were collected from each plant (4 samples per treatment) on the 20th August (first sampling) and 11th October (last sampling) to compare the elemental compositions at different sampling periods and to understand their associations with BER incidence. The fruit calyx, placenta and seeds were removed, and the fruit pericarp and leaves were dried in an oven at 70°C for seven days, after which they were weighed and ground for mineral analysis.

Mineral analysis

Pericarp and leaf samples were digested in 2 mL of 61% (w/v) HNO₃ (EL grade; Kanto Chemical, Tokyo, Japan) at 110°C in a DigiPREP apparatus (SCP Science, Canada) for approximately 2 h until the solution almost disappeared. After cooling, 0.5 mL of H₂O₂ (semiconductor grade; Santoku Chemical, Tokyo, Japan) was added and the samples were heated at 110°C for 20 min. After digestion, the tubes were cooled and filled with 10 mL of 2% (w/v) HNO₃ in Milli-Q water. The elements P, K, Ca, Mg, Fe, Mn, Zn, Cu, B, Mo, S, and Na were analyzed using inductively coupled plasma mass spectrometry (ICP-MS) (ELAN DRC-e; Perkin Elmer, Waltham, MA, USA). The total N concentration in the plant samples was determined by micro-Kjeldahl digestion.

Statistical analyses

All statistical analyses were performed with R version 3.6.3 (R Core Team, 2020).

Results

Total fresh weights and incidence of BER in ripe paprika fruits

Total fresh weights of ripe paprika fruits grown under different KNO₃ concentrations at 25°C and 30°C are shown in Figure 1. Two-way ANOVA indicated that total fresh weights were significantly affected by KNO₃ concentrations (F = 53.84, P < 0.001) and temperature conditions (F = 14.84, P = 0.001), but not by their inter-
action ($F = 2.064, P = 0.156$). Subsequent one-way ANOVAs indicated that total fresh weights significantly reduced with increasing KNO$_3$ concentrations both at 25°C ($F = 20.24, P < 0.001$) and 30°C ($F = 172.3, P < 0.001$) (Fig. 1). Total fresh weights were significantly different at 25°C and 30°C without the addition of KNO$_3$ (0 mM), but not with the addition of KNO$_3$ (27.9 and 74.4 mM).

Two-way ANOVA indicated that the incidence of BER in all fruits sampled in the last sampling period was significantly affected by KNO$_3$ concentrations ($F = 11.39, P < 0.001$), temperature conditions ($F = 10.42, P < 0.01$) and their interaction ($F = 11.12, P < 0.001$). Subsequent one-way ANOVAs showed a significant increase in the incidence of BER with increasing KNO$_3$ concentrations at 25°C ($F = 15.93, P = 0.001$), and the incidence of BER was affected by KNO$_3$ concentrations at 30°C ($F = 8.438, P = 0.009$), with the highest incidence at 27.9 mM KNO$_3$ (Fig. 2). The incidence of BER was significantly different at 25°C and 30°C with the addition of 0 mM and 27.9 mM KNO$_3$, but not with 74.4 mM KNO$_3$.

**Elemental concentrations in paprika plants**

Tables 1 and 2 show the effects of KNO$_3$ concentrations and temperature conditions on the elemental concentrations in the first and last sampled paprika fruit pericarps, respectively. Most elemental concentrations in the pericarps of the first and/or last sampled paprika were significantly affected by KNO$_3$ concentrations. All elemental concentrations (except S) in the first sampled,

**Table 1.** Elemental concentrations in the fruit pericarps of the first sampled paprika in different KNO$_3$ concentrations of cultural solution under 25 or 30°C conditions.

| KNO$_3$ concentrations (mM) | Temperature ($^\circ$C) | N (mg g$^{-1}$) | P (mg g$^{-1}$) | K (mg g$^{-1}$) | Ca (mg g$^{-1}$) | Mg (mg g$^{-1}$) | Fe (µg g$^{-1}$) | Mn (µg g$^{-1}$) | Zn (µg g$^{-1}$) | Cu (µg g$^{-1}$) | B (µg g$^{-1}$) | Mo (µg g$^{-1}$) | S (µg g$^{-1}$) | Na (µg g$^{-1}$) |
|---------------------------|------------------------|----------------|---------------|---------------|----------------|----------------|----------------|----------------|----------------|----------------|---------------|----------------|--------------|----------------|
| 0                         | 25                     | 22.02          | 4.40          | 41.15         | 1.52           | 1.56           | 47.72          | 31.02          | 28.41          | 10.96          | 2.69          | 0.96          | 3.02         | 45.23          |
| 30                        | 26.84                  | 3.90          | 42.66         | 1.82          | 1.33           | 39.72          | 32.16          | 38.91         | 11.24          | 21.74         | 0.74          | 2.69          | 70.74        |
| 27.9                      | 25                     | 22.26          | 4.65          | 51.40         | 1.33           | 1.68           | 49.46          | 29.27          | 28.65          | 9.60          | 1.33          | 1.68          | 49.46        | 29.27          |
| 30                        | 27.63                  | 4.32          | 53.32         | 1.52          | 1.52           | 49.03          | 33.56          | 48.74         | 14.85          | 17.94         | 0.97          | 3.22          | 54.06        |
| 74.4                      | 25                     | 26.02          | 4.73          | 53.80         | 1.00           | 1.57           | 50.78          | 26.19          | 30.15          | 12.38         | 1.21          | 3.04          | 48.10        |
| 30                        | 32.79                  | 4.58          | 60.91         | 1.42          | 1.48           | 46.60          | 28.74          | 50.06         | 14.44          | 14.71         | 1.12          | 3.23          | 74.36        |

**ANOVA**

| KNO$_3$ (KN) | Temperature (T) | KN×T |
|-------------|----------------|------|
| ***         | ***            | **   |
| ***         | ***            | **   |
| ***         | ***            | **   |
| ***         | ***            | **   |
| ***         | ***            | **   |
| ***         | ***            | **   |
| ***         | ***            | **   |
| NS          | NS             | **   |
| NS          | NS             | **   |
| NS          | NS             | **   |
| NS          | NS             | **   |

$^a$ NS, not significant; asterisks, significant differences at $***P<0.001$, $**P<0.01$, $*P<0.05$ by two-way ANOVA ($n = 4$).

$^b$ Different letters in a column indicate significant differences at $P<0.05$ using Tukey’s multiple comparison test following a one-way ANOVA test.
but only S and Na concentrations in the last sampled, paprika fruit pericarps were also significantly affected by temperature conditions. Significant combined effects of KNO₃ concentrations and temperature conditions were detected on the concentrations of Zn, Cu, and S in the first sampled, but only on the concentration of P in the last sampled, paprika fruit pericarps.

Most elemental concentrations in the leaves of the first (except for N and Cu) and last (except for Mo and Na) sampled paprika were significantly affected by KNO₃ concentrations (Tables 3 and 4). The concentrations of Ca, Mg, Mn, Cu, B, and S in the last sampled leaves and those of K, Ca, Fe, Mn, and S in the last sampled leaves were significantly affected by temperature conditions. Significant combined effects of KNO₃ concentrations and temperature conditions were detected on the concentrations of K, Cu, and B in first sampled, and on the concentrations of P and K in last sampled, paprika leaves.

### Principal component analysis (PCA)

To compare the profiles of 11 mineral elements (P, Ca, Mg, Fe, Mn, Zn, Cu, B, Mo, S, and Na) and the incidence of BER in the first (Fig. 3A) and last sampled (Fig. 3B) paprika fruit pericarps among treatments, PCA was used with the normal values for the concentration of each element and the incidence of BER. The score plot of PCA in the first sampled paprika fruit pericarps showed that the different KNO₃ concentrations and temperature conditions were clearly separated along axis 1, which explained 39.3% of the total variance, and axis 2, which explained 26.5%, respectively. The score plot of PCA in the last sampled pericarps showed that the different KNO₃ concentrations were separated along axis 2, which explained 26.6% of the total variance, but separation along the axis 1, which explained 45.5% of the total variance, was ambiguous.

### Table 2. Elemental concentrations in the fruit pericarps of the last sampled paprika in different KNO₃ concentrations of cultural solution under 25 or 30°C conditions.

| KNO₃ concentrations (mM) | Temperature (°C) | N (mg·g⁻¹) | P (mg·g⁻¹) | K (mg·g⁻¹) | Ca (mg·g⁻¹) | Mg (mg·g⁻¹) | Fe (μg·g⁻¹) | Mn (μg·g⁻¹) | Zn (μg·g⁻¹) | Cu (μg·g⁻¹) | B (μg·g⁻¹) | Mo (μg·g⁻¹) | S (μg·g⁻¹) | Na (μg·g⁻¹) |
|-------------------------|------------------|------------|------------|------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| 0                       | 25               | 26.87      | 43.3 b     | 42.25      | 2.04        | 2.00        | 54.18       | 47.02       | 22.93       | 12.57       | 24.86       | 0.54        | 2.78        | 34.91       |
|                         | 30               | 29.85      | 4.84 ab    | 39.83      | 2.13        | 2.02        | 56.34       | 44.08       | 28.16       | 14.93       | 29.21       | 0.56        | 2.72        | 102.01      |
| 27.9                    | 25               | 31.77      | 5.30 ab    | 50.11      | 2.53        | 2.64        | 68.52       | 51.96       | 33.86       | 13.63       | 24.05       | 0.91        | 3.25        | 16.25       |
|                         | 30               | 28.29      | 4.20 b     | 51.11      | 1.63        | 1.82        | 50.44       | 38.53       | 26.45       | 11.46       | 19.95       | 0.62        | 2.37        | 31.98       |
| 74.4                    | 25               | 42.09      | 5.76 a     | 56.25      | 1.10        | 1.75        | 56.64       | 32.95       | 31.89       | 13.98       | 14.14       | 0.65        | 2.84        | 40.98       |
|                         | 30               | 46.03      | 6.21 a     | 58.07      | 1.10        | 1.72        | 56.27       | 32.95       | 33.06       | 16.63       | 18.3        | 0.8         | 3.04        | 100.73      |

ANOVA²

| KNO₃ (KN) | Temperature (T) | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
|-----------|-----------------|----|----|----|----|----|----|----|----|----|----|----|----|----|
| 25        | 25               | *** | ** | *** | * | NS | NS | * | NS | * | ** | NS | NS | NS |
| 30        | 30               | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |

³ NS, not significant; asterisks, significant differences at ***P<0.001, **P<0.01, *P<0.05 by two-way ANOVA (n=3-4).

### Table 3. Elemental concentrations in the leaves of the first sampled paprika in different KNO₃ concentrations of cultural solution under 25 or 30°C conditions.

| KNO₃ concentrations (mM) | Temperature (°C) | N (mg·g⁻¹) | P (mg·g⁻¹) | K (mg·g⁻¹) | Ca (mg·g⁻¹) | Mg (mg·g⁻¹) | Fe (μg·g⁻¹) | Mn (μg·g⁻¹) | Zn (μg·g⁻¹) | Cu (μg·g⁻¹) | B (μg·g⁻¹) | Mo (μg·g⁻¹) | S (μg·g⁻¹) | Na (μg·g⁻¹) |
|-------------------------|------------------|------------|------------|------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| 0                       | 25               | 21.27      | 3.67       | 91.29 c    | 23.04       | 6.32        | 97.62       | 208.66      | 75.68       | 8.14 b      | 78.91 b     | 1.04        | 5.09        | 21.25       |
|                         | 30               | 19.10      | 3.10       | 76.91 c    | 18.04       | 4.95        | 106.08      | 160.84      | 94.12       | 9.09 b      | 121.33 a    | 0.91        | 4.31        | 30.52       |
| 27.9                    | 25               | 22.99      | 2.90       | 119.22 b   | 11.39       | 4.13        | 77.9        | 135.77      | 58.74       | 5.13 c      | 55.08 c     | 1.43        | 5.73        | 14.87       |
|                         | 30               | 20.70      | 3.04       | 118.64 b   | 7.46        | 2.67        | 97.76       | 111.48      | 70.12       | 12.45 a     | 55.94 c     | 1.21        | 5.68        | 8.86        |
| 74.4                    | 25               | 22.91      | 2.24       | 148.51 a   | 4.47        | 1.80        | 69.02       | 72.84       | 89.22       | 9.20 b      | 42.84 c     | 1.29        | 5.64        | 12.42       |
|                         | 30               | 22.66      | 2.15       | 169.35 a   | 3.46        | 1.66        | 66.71       | 58.45       | 82.51       | 10.22 ab    | 40.53 c     | 1.46        | 4.71        | 15.90       |

ANOVA²

| KNO₃ (KN) | Temperature (T) | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
|-----------|-----------------|----|----|----|----|----|----|----|----|----|----|----|----|----|
| 25        | 25               | *** | *** | *** | *** | *** | *** | *** | NS | *** | *** | ** | NS | NS |
| 30        | 30               | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |

³ NS, not significant; asterisks, significant differences at ***P<0.001, **P<0.01, *P<0.05 by two-way ANOVA (n=4).

Different letters in a column indicate significant differences at P<0.05 using Tukey’s multiple comparison test following a one-way ANOVA test.
Correlation coefficient analysis

Correlation coefficients between the concentrations of 13 elements and the incidence of BER in the first and last sampled fruit pericarps of paprika are shown in Table 5. Positive correlation coefficients in fruit pericarps were observed for N, K, Zn, and Cu in the first sampled and for K in the last sampled pericarps, whereas negative correlation coefficients in fruit pericarps were observed for B in the first sampled, and for Ca, Mg, Fe, Mn, B, and S in the last sampled pericarps.

Correlation coefficients between elemental concentrations in the pericarps and leaves of the first and last sampled paprika were also calculated (Tables S1 and S2). Significant correlations were found between several combinations of elements with a tendency towards relatively stronger correlations in the leaves than in the pericarps.

Table 4. Elemental concentrations in the leaves of the last sampled paprika in different KNO₃ concentrations of cultural solution under 25 or 30°C conditions.

| KNO₃ concentrations (mM) | Temperature (°C) | N (μg·g⁻¹) | P (μg·g⁻¹) | K (μg·g⁻¹) | Ca (μg·g⁻¹) | Mg (μg·g⁻¹) | Fe (μg·g⁻¹) | Mn (μg·g⁻¹) | Zn (μg·g⁻¹) | Cu (μg·g⁻¹) | B (μg·g⁻¹) | Mo (μg·g⁻¹) | S (μg·g⁻¹) | Na (μg·g⁻¹) |
|-------------------------|----------------|-----------|---------|---------|----------|---------|---------|----------|---------|--------|--------|--------|--------|---------|
| 0                       | 25             | 48.72     | 3.68 b  | 80.57 d | 16.09    | 4.24    | 89.02   | 198.51   | 67.94   | 10.56  | 134.2 | 0.78   | 5.39   | 18.08   |
|                         | 30             | 49.58     | 5.08 a  | 75.91   | 12.83   | 3.55    | 88.4    | 151.45   | 54.58   | 12.27  | 130.55 | 1.02   | 4.88   | 32.7    |
| 27.9                    | 25             | 44.06     | 3.74 b  | 104.03 c| 6.17    | 2.58    | 88.9    | 116.04   | 61.61   | 8.61   | 61.28 | 1.02   | 5.22   | 18.81   |
|                         | 30             | 49.82     | 2.95 bc | 104.48 c| 5.37    | 2.44    | 67.73   | 99.11    | 42.23   | 9.72   | 74.12 | 0.69   | 3.79   | 11.36   |
| 74.4                    | 25             | 46.49     | 2.30 c  | 133.78 b| 2.81    | 1.23    | 58.54   | 56.12    | 39.72   | 7.91   | 40.66 | 0.67   | 3.01   | 15.37   |
|                         | 30             | 42.34     | 1.76 c  | 188.89 a| 2.26    | 1.18    | 45.2    | 53.2     | 27.27   | 7.24   | 49.62 | 0.65   | 2.25   | 34.21   |

ANOVA

KNO₃ (KN) ** *** *** *** *** *** *** *** *** NS *** NS
Temperature (T) NS NS *** *** *** *** *** *** *** *** NS NS
KN × T NS ** *** NS NS NS NS NS NS NS NS NS NS

z NS, not significant; asterisks, significant differences at ***P<0.001, **P<0.01, *P<0.05 by two-way ANOVA (n=3–4).

Different letters in a column indicate significant differences at P<0.05 using Tukey’s multiple comparison test following a one-way ANOVA test.

Table 5. Correlation coefficient between elemental concentrations and the incidence of BER in the fruit pericarps of the first and last sampled paprika.

| Sampling | N     | P     | K     | Ca    | Mg    | Fe    | Mn    | Zn    | Cu    | B     | Mo    | S     | Na    |
|----------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| First    | 0.541 | 0.253 | 0.509 | −0.300| 0.030 | 0.373 | −0.009| 0.456 | 0.691 | −0.580| 0.068 | 0.352 | 0.096 |
| Last     | 0.196 | −0.015| 0.576 | −0.572| −0.504| −0.434| −0.623| 0.030 | −0.189| −0.627 | −0.085| −0.470| 0.036 |

z Asterisks, significant differences at ***P<0.001, **P<0.01, *P<0.05.
Ratios of Ca and B concentrations between leaves and fruit pericarp

The leaf to fruit pericarp ratios of Ca concentrations are shown in Figure 4A and those of B concentrations are shown in Figure 4B. The leaf/fruit ratio of Ca concentrations decreased significantly with increasing concentrations of KNO$_3$ and increasing temperature, whereas that of B concentrations tended to decrease significantly with increasing concentrations of KNO$_3$ but increased significantly with increasing temperature. The leaf/fruit ratio of Ca concentrations in the last sampled paprika was lower than that in the first sampled paprika in all treatments, the percent decrease ranging from 15.4 to 70.5%, while the leaf/fruit ratio of B concentrations increased in three treatments (0 mM KNO$_3$ at 25°C, 27.9 mM KNO$_3$ at 30°C, and 74.4 mM KNO$_3$ at 25°C), the percent decrease ranging from 17.5 to 50.8%, and decreased in the other treatments, the percent decrease ranging from 0.6 to 13.6%.

![Graph](A)

Fig. 4. The leaf/fruit ratios of the concentrations of calcium (A), and boron (B) in the fruit pericarps of the first sampled (white bars) and last sampled (gray bars) paprika in different KNO$_3$ concentrations of cultural solution under 25 or 30°C conditions. Vertical bars indicate ± SE (n = 3–4). Different letters indicate significant difference at P < 0.05 using Tukey’s multiple comparison test following a one-way ANOVA test for the same temperature conditions.

### Discussion

**Relationship between potassium nitrate concentration and temperature, and total fresh weight and the incidence of BER**

Application of high concentrations of KNO$_3$ significantly reduced total fresh weight and increased the incidence of BER in paprika fruits grown in rockwool culture both at 25°C and 30°C (Figs. 1 and 2), demonstrating that N and K concentrations above 46.5 mM and 36.5 mM, respectively, were likely to reduce the marketable yield. Similarly, it has been reported that N fertilizer at a rate greater than 70 kg/ha reduces the yield of pepper plants growing in soil (O’Sullivan, 1979), whereas other reports showed that the threshold rate of N fertilizer that reduced the marketable yield was quite wide at 134–336 kg/ha (Thomas and Heilman, 1964; Iley and Ozaki, 1967; Locascio and Fiskell, 1977). In soilless growth medium, N concentrations of 8.3–9.3 mM have been reported to increase pepper yield and quality (Bar-Tal et al., 2001), while in other locations a 15.5 mM N concentration is required (Savvas et al., 2008). As these experiments were conducted in different regions, it is likely that they were influenced by temperature and other factors such as climate and irrigation rate. High temperatures reduce the concentration of reducing sugars in the flower buds and flowers of peppers, leading to reduced fruit set (Aloni et al., 1991; Erickson et al., 2001) and directly impacting fruit yield (Kawasaki and Yoneda, 2019). This indicates a difference in total fresh weight of paprika fruits at different temperatures (Fig. 1). High temperature also increases the production of reactive oxygen species (ROS) in the fruit, leading to cell death and BER incidence (Saure, 2014). Since ROS production is also increased by salinity stress (Aktas et al., 2005), ROS may be one of the reasons for the differences in the incidence of BER between temperature and KNO$_3$ concentrations (Fig. 2). Although there are few reports on the upper limit of potassium fertilization in paprika, the ratio of K to N has been reported to be important in habanero pepper (Capsicum chinense Jacq.) production; a ratio of greater than 1 reduces production (Medina-Lara et al., 2008). In the present study, we found that the total fresh weight and the incidence of BER in paprika fruits in response to different KNO$_3$ concentrations were different at 25°C and 30°C, suggesting that temperature is one of the controlling factors that determine the threshold of N and K fertilizers to maintain a marketable paprika yield. The use of N and K fertilizers needs to be modified based on local temperature conditions to maximize the production and quality of paprika.

**Elements related to the incidence of BER**

We investigated changes in the elemental composition of paprika fruit pericarps grown under different...
KNO₃ concentrations and different temperature conditions, and their relationship to the incidence of BER. The elemental composition of the first and last sampled fruit pericarps was affected by increasing concentrations of KNO₃ (Tables 1 and 2). Notably, the B concentration in paprika pericarps was negatively affected by increasing concentrations of KNO₃, and negative correlations were detected between B concentrations of both the first and last sampled pericarps and the incidence of BER (Fig. 3; Table 5). B induces the crosslinking of pectin in the cell wall, and is mainly xylem mobile, as is Ca (Raven, 1980). Pectic networks are formed by the crosslinking of pectic polysaccharide chains, and these crosslinks are established in two different domains of pectin: homogalacturonan (HG) by Ca divalent cations and rhamnogalacturonan II (RG-II) by borate (Funakawa and Miwa, 2015). Borate forms a network of pectic polysaccharides in cell walls by crosslinking two chains of pectic polysaccharides in the RG-II region through borate-diester bonding (Matoh, 1997).

In this study, fruit pericarps may have been deficient in B from the early growth stage under the influence of KNO₃, leading to the destabilization of pectin-B crosslinking and subsequent BER occurrence. In addition, it is suggested that RG-II, which comprises less than 10% of pectin (O’Neill et al., 2004), is much more critical for BER occurrence than HG, which comprises about 65% of pectin (Mohnen, 2008). Alternatively, the pectin in the paprika pericarp may contain a greater proportion of RG-II.

Studies have shown that foliar application of B reduces Ca deficiency symptoms in apple fruit (Dixon et al., 1973) and concentric and radial cracks in tomato fruit (Davis et al., 2003). The involvement of B in BER in tomatoes has also been investigated, and it has been reported that spraying with Ca and B reduced the incidence of BER (Liebisch et al., 2009). However, as the authors themselves noted, this study did not directly demonstrate whether the reduction in BER incidence was attributable to Ca or B. The present study also does not directly demonstrate that B deficiency in the fruit pericarp is a factor in the incidence of BER, and thus further studies such as B addition and deficiency experiments are needed.

BER is widely considered to be caused when the supply of Ca is insufficient to meet the requirements of the fruit (Taylor and Locascio, 2004). In this study, no correlation was detected between the incidence of BER and the concentrations of Ca in the first sampled fruit pericarps (Fig. 3; Table 5), and the leaf/fruit ratios of Ca concentrations in paprika pericarps reduced significantly with increasing KNO₃ concentration at a temperature of 30°C (Fig. 4). Although leaves generally have a higher transpiration rate, and therefore a greater supply of Ca than fruits (Ho and White, 2005; De Freitas et al., 2011; De Freitas and Mitcham, 2012), excessive KNO₃ addition, as well as high temperature stress, contrary to our hypothesis may have inhibited transpiration flow to the leaves rather than closing the stomata, resulting in a relatively high Ca transport towards the fruit, resulting in no significant correlation of Ca concentration with BER incidence. The leaf/fruit ratios of Ca concentrations in the last sampled paprika pericarps decreased, but the decrease was smaller in the pericarps of paprika plants grown at 30°C with 27.9 mM KNO₃ and 74.4 mM KNO₃ concentrations than that in the first sampled paprika pericarps (Fig. 4). It is well established that the addition of KNO₃ increases the electrical conductivity (EC) in the nutrient solution. High EC levels reduce the water potential in the root zone and water uptake (Li and Stanghellini, 2001; Suzuki et al., 2015), which is likely to affect the translocation, localization or distribution of Ca²⁺ (Kataoka et al., 2017) and increase the incidence of BER in tomato fruits (Ho and White, 2005). Our and previous results indicate that, as cultivation progressed, Ca uptake from the roots may have been inhibited by high KNO₃ concentrations and high temperatures, which may have prevented Ca transport to the fruits, resulting in no significant correlation between Ca concentration and BER incidence in the first, but not in the last, sampled paprika pericarps (Table 5).

However, for B, which is transported via transpiration similar to Ca, the profiles of the B concentrations and leaf/fruit ratios in each treatment were different from those of Ca, implying that either Ca or B may have other distribution mechanisms in addition to transpiration flow. It has been reported that B partially moves into the phloem with sorbitol in fruit trees (Kanayama, 2009) and celery (Hu et al., 1997). Although there have been no reports of sorbitol synthesis in paprika, it is assumed that B may be transported not only by the xylem, but also the phloem through a similar mechanism. Consequently, when nutrient uptake from the roots is reduced by the addition of KNO₃, the Ca concentration in the fruit may be maintained to some extent via reduction of the leaf/fruit ratio of the Ca concentration, whereas the B concentration in the fruit may be reduced as a result of maintenance of the leaf/fruit ratio, which may have an effect on BER incidence. In addition, as growth progressed, the supply of Ca decreased, which may have contributed to BER incidence, as the requirement of the fruit may not have been maintained by adjustment of only the leaf/fruit ratio.

Similarly to Ca, Mg concentrations in the paprika pericarps showed no significant correlation with the incidence of BER in the first sampled, but did so in the last sampled pericarps (Fig. 3; Table 5). Mg concentration in the last sampled paprika pericarp was not significantly affected by either KNO₃ concentration or temperature (Table 2), whereas that in the leaves was significantly affected by KNO₃ concentration (Table 4). In tomatoes, salinity is likely to reduce Mg uptake,
resulting in a reduction in Mg concentration in both fruits and leaves (Kataoka et al., 2017). Since Mg$^{2+}$ is a phloem mobile nutrient (Bukovac and Wittwer, 1957), the Mg concentration in paprika fruit may be maintained at a constant level by retranslocation from the leaves. Although it has been reported that an increase in Mg in the fruit results in a relative decrease in Ca concentration, resulting in BER (Tadesse et al., 2001), Mg concentration in the last sampled pericarp was significantly positively correlated with the Ca concentration, while the first sampled pericarp showed a significant negative correlation (Tables S1 and S2). This implies that Mg is not directly involved in BER incidence, but may have indirectly appeared to have a negative correlation by behaving similarly to Ca. However, it is unclear why Mg and Ca showed opposite effects between the first and last sampled pericarp, and further investigation is needed.

Mn concentrations in the paprika pericarps also showed a significant correlation with BER incidence only in the last sampled pericarp (Fig. 3; Table 5). Mn is an element involved in protection against ROS production (Kawano et al., 2002; Abreu and Cabelli, 2010). Peppers grown under high salinity conditions have been reported to have significantly reduced Mn concentrations in fruits and leaves, as well as increased BER incidence (Aktas et al., 2005). These results indicate that, as cultivation progresses, ROS production in plants increased with a high concentration of KNO$_3$ and high temperature, whereas plants that could not increase Mn uptake caused by a high concentration of KNO$_3$ may not eliminate ROS in the fruit, resulting in increased BER.

As the concentration of KNO$_3$ increased, the N concentration in the fruit pericarps increased (Tables 1 and 2), and there was a significant correlation between the N concentration of the first sampled pericarps and the BER incidence (Table 5), suggesting that the increased N concentration enhanced fruit expansion and increased the probability of BER occurrence (Marcelis and Ho, 1999). In contrast, it has been demonstrated that capsaicin concentration in habanero pepper fruit fluctuates with increasing N addition (Medina-Lara et al., 2008), and the biosynthesis of secondary metabolites using N may also increase, which may explain why there was no significant correlation between the N concentration in the last sampled pericarp and the BER incidence.

Correlation coefficient analysis revealed that the K concentration of paprika fruits was also related to the incidence of BER (Table 5). K is involved in the regulation of osmotic pressure for the transport of substances in plants, including photoassimilate transport to fruits (Marcelis and Ho, 1999). Retranslocation of K to the fruits promotes the transport of assimilation products to them and promotes fruit growth, suggesting that excess K transport may disrupt the balance between growth and Ca transport to the fruit, resulting in BER occurrence (Marcelis and Ho, 1999). In addition, it has been reported that as salinity increases, the Ca and Mg levels of the same charge decrease, causing BER (Adams and Ho, 1993). In this study, as the K concentration increased, the Ca and Mg concentrations in the leaves significantly decreased, whereas those in the fruits were not significantly correlated with K concentrations (Tables S1 and S2). These observations suggest that the elemental dynamics in the paprika fruit pericarp cannot be explained only by charge balance, and that another mechanism may be involved.

Conclusion
The present study demonstrated the importance of adjusting N and K fertilizer inputs based on temperature by growing paprika plants under different KNO$_3$ concentrations and temperature conditions. It has been shown that Ca deficiency may not be the only reason for BER (Nonami et al., 1995) and that elements other than Ca, i.e., several elements at different concentrations are involved in BER incidence. An ionomic approach to fruits provides a more accurate assessment of elemental dynamics. Further identification of the elemental composition involved in BER incidence will provide the ionome, and this will help in the development of a tool to predict the BER incidence in paprika. This may contribute to stabilizing crop production by improving fertilizer application and environmental control.

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