Effects of feeding leaf positions on the growth and fruit quality in muskmelon plants showing leaf yellowing symptoms

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Abstract: This study was conducted to evaluate the influence of feeding leaf positions on the growth, net formation of fruits, and occurrence of leaf yellowing symptoms (LYS) in muskmelon plants. Plants having five or ten more leaves above the fruit-bearing node produced the greater biomass than those of plants having equal or five less leaves above the fruit-bearing node. The number of leaves above the fruit-bearing node also influenced on the occurrence of LYS. The number of plants with LYS decreased as the number of leaves borne on the nodes above the fruit-bearing node increased. The LYS infected ratio of BL-5 treatment were the greatest, while fruit weight of BL+5 treatment were the greatest among all the tested treatments. In addition, the net formation of BL-5 treatment showed the poorest. Results indicated that maintaining the higher number of leaves over the fruit-bearing node might be feasible the practical method for coping physiological damages from yellowing symptoms.

Key words: Infected plant ratio, Leaf number, Net index, Photosynthetic rate, Root activity, Fruit-bearing node

I. Introduction

Muskmelon is one of the major fruit bearing vegetables in Korea where its cultivation area and production have increased from 17,000 metric tons (659 ha) in 2,000 to 48,000 tons (1,477 ha) in 2013 (MIAFRA, 2014a). About 1,100 tons of muskmelon were exported to Japan and other countries in Southeast Asia in 2013 (MIAFRA, 2014b). Due to recent advances in muskmelon cultivation technology, growers now produce high quality muskmelon in greenhouses all year-round. Nevertheless, there are still low quality muskmelon with low sugar contents and poorly netted skins which depreciate their commercial values.

The major reason for low quality muskmelon in these days is the occurrence of LYS (Fig. 1). In the past 2 to 3 years, LYS on muskmelon has steadily increased in all production areas in Korea. However, the cause of LYS has not been identified. Affected plants showed light green spots on the surface of the lower leaves (sometimes upper leaves) at the beginning and then gradually developed LYS. The yellow spots on the leaf were enlarged until a whole leaf blade was changed to yellow color and, finally, LYS spread to upper leaves. In the plants with LYS, normal fruit development were retarded with little netting on the fruit skin and low sugar contents (Lee et al., 2009; Park et al., 2011).

Plants showing LYS were reported to have unbalanced sugar translocation from the leaves, fewer roots, larger leaves, and faster fruit enlargement compared to normal plants especially when grown under poor environmental conditions (Takeshita, 2004). The objective of this study was to characterize the influence of cultural practices aimed at minimizing LYS in muskmelon plants.
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II. Materials and methods

Seeds of a muskmelon cultivar ‘Earl’s Tipani’ (Hannil Seed Co., Gongju, Korea) were sown in 32-cell plug trays (280 × 540 × 63 mm, W × L × H, Bumnong Co., Ltd., Seoul, Korea), respectively, filled with a commercial root substrate (BM2, Berger Group Ltd., Quebec, Canada) in a greenhouse. Seedlings at the two true-leaf stage were transplanted into plastic-mulched raised beds (plant spacing 40 cm) in greenhouses at National Institute of Horticultural and Herbal Science, Suwon, Korea on June 4, 2014. Female flowers formed on the secondary vines were pollinated for fruit set. Out of three developing fruits from pollinated female flowers borne on the secondary vines growing from the 11th to 13th nodes, only one fruit with good shape is kept with the rest of the developing fruits being removed after 7 to 10 days from pollination.

Plant growth, fruit yield and quality, and LYS development were examined with the influence of the position of the leaves borne above and below the fruit-bearing node (FBN). Four treatments were used were BL−5 (five leaves less above the fruit-bearing node than below it), BL=0 (same number of leaves left above and below the fruit-bearing node), BL+5 (five more leaves were kept above the fruit-bearing node than below it), and BL+10 (ten more leaves were kept above the fruit-bearing node than below it).

Net photosynthetic rates of the leaf were measured on six plants (two plants with three replications) on each treatment using a portable photosynthesis system (LI-6400, LI-COR, Lincoln, NE, USA). The fully expanded leaves borne on the 5th to 7th nodes from the terminal bud were used to measure net photosynthetic rate at 75 days from transplanting. Flow rate, CO$_2$ concentration, and photosynthetic photon flux density were maintained at 500 µmol·mol$^{-1}$, 400 µmol·mol$^{-1}$, and 1,000 µmol·m$^{-2}$·s$^{-1}$, respectively. Leaf temperature was also set at 25°C and RH at 60%. Root activities, as estimated by absorbance values of formazan products, were measured for six plants (two plants with three replications) at 35 days after fruit set. Roots were sampled from transplanting positions at 50 cm in radius and 30 cm in depth, and washed in running water before measuring. Fine roots were cut into 0.5 cm and homogenized, and then 0.1 g of homogenized root sample was added in a test tube with 1 mL deionized water and mixed with 10 µL of WST-1 assay kit (Premix WST-1 cell proliferation assay system, Takara Bio Inc., Otsu, Japan). Mixed samples were placed in the dark at 25°C for 3 h, and analyzed by ELISA reader (Microplate Spectrophotometer, EonTM, BioTek Inc., Winooski, VT, USA) at 420 nm.

All the measurements were replicated three times. In root pruning and seedling age experiments, data of plant growth, photosynthetic rate, chlorophyll content, and root activity were analyzed by using $t$-test ($P < 0.05$). For other measurement, experimental data were analyzed by using Duncan’s multiple range
test at $P < 0.05$ in SAS 9.2 (SAS Inst. Inc., Cary, NC, USA) to identify least significant differences ($P < 0.05$).

### III. Results and discussions

The influence of the number and the position of the leaves on growth of muskmelon plants were investigated 75 days after transplanting (Table 1). The more number of feeding leaves above the fruit-bearing node on the vine, the greater the plant height, root fresh and dry weights, shoot fresh and dry weights. The greatest plant growth was found in the BL+10 treatment, followed by the BL+5 treatment, the BL=0 treatment, and the BL−5 treatment. The plant height and shoot fresh weight were greatest (234.5 cm and 904.9 g) in the BL+10 treatments, followed by the BL+5 (207.8 cm and 714.4 g), BL=0 (164.0 cm and 619.7 g), and BL−5 (123.8 cm and 473.8 g) treatments. Plants having five or ten more leaves above the fruit−bearing node produced the greater biomass with greater fresh and dry weights of shoots and roots than plants having equal or five less leaves above the fruit−bearing node. The greater the number of the feeding leaves placed above the fruit−bearing node, the higher the shoot weights compared to the plants having more foliage below the fruit−bearing node. Those results were similar with previous research that pinching position, above the fruit−bearing node, improved leaf area of muskmelon plants (Kang, 2010).

The effect of leaf number above and below the fruit−bearing node on photosynthesis, root activity, and incidence of LYS was investigated 75 days after transplanting (Table 2). The plants in the BL+5 treatment resulted in significantly smaller photosynthetic rate and chlorophyll content (13.5 µmol CO$_2$·m$^{-2}$·s$^{-1}$ and 45.6 in SPAD value) compared to those of other treatments. But, the root activity was greatest in the plants in BL=0 and BL+5 treatments (0.38 and 0.40 in absorbance values of formazan products, respectively), followed by the BL−5 and BL+10 treatments (0.23 and 0.31, 45.0 ± 5.0)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plant height (cm)</th>
<th>Leaf area (cm$^2$)</th>
<th>Root fresh weight (g)</th>
<th>Root dry weight (g)</th>
<th>Shoot fresh weight (g)</th>
<th>Shoot dry weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL−5$^1$</td>
<td>123.8 d$^2$</td>
<td>6,241 a</td>
<td>14.5 bc</td>
<td>1.24 b</td>
<td>473.8 d</td>
<td>38.0 c</td>
</tr>
<tr>
<td>BL=0</td>
<td>164.0 c</td>
<td>8,000 a</td>
<td>12.9 c</td>
<td>1.22 b</td>
<td>579.6 c</td>
<td>46.5 bc</td>
</tr>
<tr>
<td>BL+5</td>
<td>207.8 b</td>
<td>8,426 a</td>
<td>16.9 ab</td>
<td>1.50 ab</td>
<td>734.8 b</td>
<td>52.3 b</td>
</tr>
<tr>
<td>BL+10</td>
<td>234.5 a</td>
<td>9,456 a</td>
<td>19.2 a</td>
<td>1.66 a</td>
<td>904.9 a</td>
<td>72.8 a</td>
</tr>
</tbody>
</table>

$^1$BL (bottom leaves) means number of leaves under fruit-bearing node.

$^2$Mean separation within columns by LSD test at $P < 0.05$.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Net photosynthetic rate (µmol CO$_2$·m$^{-2}$·s$^{-1}$)</th>
<th>Chlorophyll contents (SPAD value)</th>
<th>Root activity$^3$ (Abs.)</th>
<th>Percentage of infected plant$^4$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL−5$^3$</td>
<td>15.1 a$^2$</td>
<td>49.0 abc</td>
<td>0.23 b</td>
<td>45.0 ± 5.0</td>
</tr>
<tr>
<td>BL=0</td>
<td>15.6 a</td>
<td>51.4 ab</td>
<td>0.38 a</td>
<td>33.1 ± 13.1</td>
</tr>
<tr>
<td>BL+5</td>
<td>13.5 b</td>
<td>45.6 c</td>
<td>0.40 a</td>
<td>25.0 ± 15.0</td>
</tr>
<tr>
<td>BL+10</td>
<td>14.2 a</td>
<td>52.5 c</td>
<td>0.31 b</td>
<td>17.5 ± 7.5</td>
</tr>
</tbody>
</table>

$^3$Mean separation within columns by Duncan’s multiple range test at $P = 0.05$.

$^2$BL (bottom leaves) means number of leaves under fruit-bearing node.

$^3$Root activity represents absorbance value of formazan product.

$^4$Data are mean ± standard error.
Table 3. Effect of number of feeding leaves above fruit-bearing node on vine on fruit quality in muskmelon plants 75 days after transplanting.

<table>
<thead>
<tr>
<th>State</th>
<th>Treatment</th>
<th>Length (cm)</th>
<th>Width (cm)</th>
<th>Shape index</th>
<th>Weight (g)</th>
<th>Net index</th>
<th>Thickness of mesocarp (cm)</th>
<th>Sugar content (°Bx)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>BL-51)</td>
<td>14.7 d 1)</td>
<td>13.9 c</td>
<td>1.05 a</td>
<td>1,497 b</td>
<td>9.0 d</td>
<td>3.5 b</td>
<td>10.7 ab</td>
</tr>
<tr>
<td></td>
<td>BL=0</td>
<td>16.5 ab</td>
<td>15.3 ab</td>
<td>1.08 a</td>
<td>2,082 a</td>
<td>4.3 a</td>
<td>4.0 a</td>
<td>11.6 a</td>
</tr>
<tr>
<td></td>
<td>BL+5</td>
<td>16.4 ab</td>
<td>15.4 a</td>
<td>1.05 a</td>
<td>2,018 a</td>
<td>6.0 bc</td>
<td>3.9 a</td>
<td>11.4 a</td>
</tr>
<tr>
<td></td>
<td>BL+10</td>
<td>17.2 a</td>
<td>16.1 a</td>
<td>1.08 a</td>
<td>2,281 a</td>
<td>4.5 ab</td>
<td>4.0 a</td>
<td>11.5 a</td>
</tr>
<tr>
<td>LYS</td>
<td>BL-5</td>
<td>12.9 e</td>
<td>11.9 d</td>
<td>1.08 a</td>
<td>896 c</td>
<td>8.8 d</td>
<td>NA 3)</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>BL=0</td>
<td>15.3 cd</td>
<td>14.4 bc</td>
<td>1.07 a</td>
<td>1,596 b</td>
<td>6.6 c</td>
<td>3.8 a</td>
<td>8.8 c</td>
</tr>
<tr>
<td></td>
<td>BL+5</td>
<td>16.0 bc</td>
<td>15.3 ab</td>
<td>1.05 a</td>
<td>1,950 a</td>
<td>6.3 c</td>
<td>3.8 a</td>
<td>9.4 bc</td>
</tr>
<tr>
<td></td>
<td>BL+10</td>
<td>17.0 ab</td>
<td>15.7 a</td>
<td>1.08 a</td>
<td>2,175 a</td>
<td>5.7 abc</td>
<td>4.1 a</td>
<td>9.4 bc</td>
</tr>
</tbody>
</table>

1) BL (bottom leaves) means number of leaves under fruit-bearing node.
2) Mean separation within columns by Duncan’s multiple range test at $P < 0.05$.
3) NA means non-available because of too small fruit size.

respectively). The incidence of LYS decreased as the number leaves above the fruit-bearing node increased, and percentage of the plants showing LYS in the BL-5 treatment (45.0%) was 1.4 times as great as that of the BL=0 treatment (33.1%). Plants in the BL+5 and BL+10 treatments showed the lower incidence of LYS (76% and 53%, respectively) compared to that of the BL=0 treatment. This result shows that more than five more feeding leaves must be kept above the fruit-bearing node than below it to reduce the incidence of LYS effectively.

Number of leaves kept above and below the fruit-bearing node also influenced on the size and quality of fruits. Length, width, and weight of fruits 75 days after transplanting significantly decreased in the BL-5 treatment regardless of LYS (Table 3). The plants not showing LYS in the BL=0, BL+5, and BL+10 treatments had the greater fruit sizes than that of the BL-5 treatment. The plants showing LYS in the BL+10 and BL+5 treatments resulted in the greater fruit sizes compared to the BL-5 treatment. There was no significant difference in fruit shape index among treatments. Net formation of the fruits in the BL-5 treatment was the smallest regardless of LYS. Fruit quality (length, diameter, weight, mesocarp thickness of fruit, soluble solid content, and net index) was affected by the increased number of leaves above the fruit-bearing node in muskmelon plants (Hwang et al., 1998; Kamitani, 1967; Shishido et al., 1992).

Fruit mesocarp thickness and sugar contents for the plants that showed LYS in the BL-5 treatment could not be measured due to too small size. It was the smallest in the plants that did not show LYS in the BL-5 treatment among other treatments. Sugar content of fruits was higher in the plants not showing LYS than that of the plants showing LYS. Among the plants showing LYS, those in the BL=0 treatment resulted in the lowest sugar content. The plants showing LYS in the BL-5 treatment resulted in the lowest fruit size and weight (length 12.9 cm, width 11.9 cm, weight 896 g). Fruit weights were significantly the lowest in the plants showing LYS in the BL-5 treatments, followed by the plants not showing LYS in the BL-5 treatment plants (1,497 g) and those showing LYS in the BL=0 treatment (1,596 g), and those of other treatments (over 2,000 g).

The greater net index of fruit was found in the plants not showing LYS in the BL=0 and BL+10 treatments (4.3 and 4.5), while that of other treatments was 5.7 regardless of LYS. The smaller number of leaves left above the fruit-bearing node negatively influenced the mesocarp thickness of fruits. There
was little difference in the mesocarp thickness of fruits when the plants had equal or the greater numbers of leaves above the fruit-bearing node than below it. The plants showing LYS in the BL=0 treatment resulted in the lowest sugar content (8.8 °Bx), followed by the plants showing LYS in the BL+5 and BL+10 treatments (9.4 °Bx both). The higher sugar contents (about 11 °Bx) were measured in fruits of the plants not showing LYS (higher than 11 °Bx). These results were consistent with previous reports that pinching treatments reduced not only plant leaf area but also fruit quality (Kang, 2010). In addition, the fruit thinning reduced yield, but influenced soluble sugar content of melon fruits (Long et al., 2004). Hence, maintaining an enough number of feeding leaves above the fruit-bearing node, not below, is a good management strategy for reducing LYS in greenhouse muskmelon cultivation (Han and Park, 1993).

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