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Chlorophyll Fluorescence Characteristics of Cucumber Grafted Seedlings Graft-taken under LED Illumination with Different Light Quality and Light Intensity

Yong Hyeon Kim and Hyeong Gon Kim, Dept. of Bioindustrial Machinery Engineering, College of Agriculture & Life Sciences, Chonbuk National University, Jeonju 561-756, Korea (Institute of Agricultural Science & Technology)

Abstract

Light environment greatly affect the graft-taking and growth of grafted seedlings. Chlorophyll fluorescence characteristics of cucumber (*Cucumis sativus* L. cv. Baekdadaki) grafted seedlings healed and acclimated in a closed transplant production system (CTPS) were analyzed in this study. Two levels (25 and 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$) of photosynthetic photon flux (PPF) and four levels (red, blue, red+blue, and white LED) of light quality were provided to investigate the effects of light intensity and light quality of LED lamps on the chlorophyll fluorescence, graft-taking, and growth of cucumber seedlings. Air temperature, relative humidity, and photoperiod in CTPS were maintained at 25℃, 90%, and 14/10 h (light/dark period), respectively. At 7 days after grafting, relative humidity was lowered to 70% and PPF was increased to 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Chlorophyll fluorescence of cucumber grafted seedlings under different PPF and light quality was measured by a portable kinetic imaging fluorometer(Handy FluorCam, Photon Systems Instruments, Czech Republic) once at every day after grafting. The variable fluorescence (Fv) for rootstock and scion of cucumber grafted seedlings were significantly affected by PPF and light quality of LED lamps. Fv for scion was the highest with red LED, while Fv significantly decreased under blue LED. Quantum yield (Fv/Fm) for scion was the greatest with blue LED. However, Fv/Fm for scion significantly decreased under red LED. Cucumber seedlings healed and acclimated under blue light with PPF of 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ showed the lowest graft-taking ratio. Leaf expansion including leaf area and leaf length was significantly decreased with blue LED illumination. From these results, it was concluded that blue light inhibited the graft-taking ratio and growth of cucumber grafted seedlings. And the chlorophyll fluorescence parameters including variable fluorescence and quantum yield can be used to evaluate the joining of scion onto rootstock of grafted seedlings.

Keywords: chlorophyll fluorescence, grafted seedlings, LED, light quality, light intensity

1 Introduction

Grafting of fruit-bearing vegetables has been widely applied to increase the resistance to soil-borne diseases, to increase the tolerance to low temperature or to soil salinity, to increase the plant vigor, and to extend the duration of economic harvest time (Lee and Oda, 2003). However, grafting requires time, space and materials and is so laborious. Also a high expertise is required for grafting, healing and acclimation.
After grafting, it is important to control the environments around grafted seedlings for the robust joining of scion and rootstock. Usually the shading materials and plastic film are used

to prevent grafted seedlings from wilting by excessive transpiration and to promote the healing of grafted plants in greenhouse or tunnel. It is quite difficult to optimally control the physical environment for healing and acclimation of grafted seedlings under natural light. Therefore the growers or managers rely on their empirical knowledge for healing and acclimation of grafted seedlings (Kim, 2000; Jang et al., 2011).

Light-emitting diodes (LED) have been used as a new artificial lighting source to promote photosynthesis (Goins et al., 1997; Tennessen et al., 1995), to control photomorphogenic responses (Brown et al., 1995; Stutte, 2009), to improve the seedling quality of vegetable transplants (Lee et al., 2012; Kim and Lee, 2004), and to enhance phytochemicals (Wu et al., 2007; Li and Kubota, 2009) due to their small mass and volume, low electric consumption, long lifetime, specific wavelength, and easy pulse drive (Barta et al., 1992).

Plants are often exposed to sudden short-term or long-term stress events which reduce cell activity and plant growth to a minimum. This can lead to a severe damage eventually causing cell death if the stress coping mechanisms or repair mechanisms of plants are overworked (Lichtenthaler, 1996). Chlorophyll fluorescence is a measure of the efficiency of photosynthesis and and electron transport reactions (Maxwell and Johnson, 2000). Thus, chlorophyll fluorescence analysis can be used to detect the physiological responses of horticultural crops to low or chilling temperature (Ahn et al., 1999; Lichtenthaler and Rinderle, 1988; Greaves and Wilson, 1987; Walker et al., 1990).

Recently, LED lamps are introduced to enhance the healing and acclimation of grafted seedlings in some nurseries in Korea. In these cases, light intensities measured on the surface of plug trays were approximately ranged of 12 to 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Even though white LED as well as red and blue LED are used as artificial lighting sources for healing and acclimation of grafted seedlings, reports on the effects of light intensity and light quality of LED lamps on the survival and growth of grafted seedlings were limited (Kim and Park, 2003; Jang et al., 2011). The purpose of this study was to analyze the chlorophyll fluorescence characteristics of cucumber grafted seedlings healed and acclimated under LED illumination with different light intensity and light quality.

2 Materials and methods

2.1 Plants and grafting

Cucumber (*Cucumins sativas* L. cv. Baekdadaki) and figleaf gourd (*Cucurbita ficifolia* Bouche) were used as scion and rootstock, respectively. They were raised in 128-cell plug trays (W 280 mm x L 540 mm x H 48 mm, Bumnong Co., Korea), filled with commercial growing mixture (BM1, Berger, Canada). Grafting was done by splice grafting method when the cotyledons of the scions and rootstocks were completely unfolded. One cotyledon and the growing point of the rootstock were removed for grafting. The scion was cut 5 mm below cotyledon. After placing the scion on the rootstock, grafting clips were used to fix the grafted position tightly together. Nutrient solution of pH 5.5-6.0 and EC 1.5 $mS \cdot cm^{-1}$ was supplied to grafted seedlings by subirrigation at 6 days and 10 days after grafting.

2.2 Healing and acclimation of cucumber grafted seedlings

A growing bed (W 1200 mm x D 660 mm x H 2000 mm) with four shelves for healing and acclimation of grafted seedlings was made of aluminum profile and was placed in a closed transplant production system (CTPS). Grafted seedlings were healed and acclimated at darkness for 24 h after grafting. And they were healed and acclimated under LED lamps with photoperiod of 14/10 h (light/dark period) for two weeks. Red, blue, red+blue (LP-RB2820-120C, L-PEC, Korea) and white (LP-W2020K45-120C, L-PEC, Korea) LED lamps were attached to every shelf in a CTPS(Fig. 1). One hundred and twenty plants (40 plants x 3 plug trays) were grafted for every treatment.

Spectral characteristics of red, blue, red+Blue, and white LED were shown in Fig. 2. Photosynthetic photon flux (PPF) illuminated from LED lamps was controlled by a LED controller (S002CTRL-8, L-PEC, Korea). Air temperature, relative humidity, and air current speed in a

CTPS were controlled with a PID controller (IPC5000, Honeywell, USA) and a variable frequency inverter (iG5A, LS Industrial Systems Co., Korea). Air temperature and relative humidity were controlled at 25°C and 90%, respectively. Two PPF levels of 25 and 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and four light quality levels of red, blue, red+blue, and white LED were provided to investiagte the the chlorophyll fluorescence characteristics of cucumber grafted seedlings graft-taken under LED illumination with different light quality. At 7 days after grafting, relative humidity was lowered to 70% and PPF was increased to 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Copper-constantan thermocouples (PP-T-24, OMEGA Engineering Inc., USA) and humidity sensors (CHS-UGS, TDK, Japan) were used to measure the air temperature and relative humidity around grafted seedlings at every treatment. The graft-taking ratio and growth characteristics, such as leaf area, fresh weight, dry weight, leaf length, and SPAD value were measured at 14 days after grafting. SPAD value was measured by a chlorophyll meter (SPAD-502, MINOLTA Co., Japan). Nine plants at every treatment were sampled. Graft-taking ratio was defined as the percentage of number of grafted seedlings survived to the number of grafted seedlings at different treatment.

2.3 Measurement of chlorophyll fluorescence

The chlorophyll fluorescence was measured by a portable kinetic imaging fluorometer (Handy FluorCam, Photon Systems Instruments, Czech Republic). The maximum fluorescence (Fm) and minimum fluorescence (Fo) of cucumber leaves fully dark-adapted were measured. Then variable fluorescence (Fv=Fm-Fo) and quantum yield (Fv/Fm) were calculated once at every day during healing and acclimation of grafted seedlings. The chlorophyll fluorescence characteristics for scion and rootstock were determined by image segmentation. Nine plants at every treatment were sampled.

2.4 Statistical analysis

Statistical analysis was conducted with the software package SAS v9.3 (SAS Institute, Cary, USA). The data were subjected to an analysis of variance (ANOVA). The differences in mean values of graft-taking, growth and fluorescence characteristics as affected by PPF and light quality of LED lamps used in this study were compared by LSD-test.

3 Results and Discussion

3.1 Chlorophyll fluorescence characteristics

Chlorophyll fluorescence characteristics for rootstock and scion of cucumber grafted seedlings as affected by PPF and light quality of LED lamps were presented in Fig. 3 and Fig. 4. Variable fluorescence (Fv) for rootstock of grafted seedlings healed under different PPF and light quality of LED lamps decreased with increasing days after grafting (Fig. 3). However, Fv for scion did not sharply decrease like those of rootstock. At 4-6 days after grafting, Fv was significantly affected by light quality of LED lamps. During this period, Fv for scion was the highest with red LED, followed by white LED, while under blue LED, Fv significantly decreased. It was known that scion onto rootstock began to join at 4-5 days after grafting. Quantum yield (Fv/Fm) for rootstock of grafted seedlings was in the range of 0.82-0.84 for PPF of 25 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and 0.82-0.86 for PPF of 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Fv/Fm gradually increased with increasing days after grafting. This result was obviously found at the treatment with PPF of 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Fv/Fm for scion was ranged of 0.80-0.84 for PPF 25 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and 0.79-0.85 for PPF 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Fig. 4). During 3-5 days after grafting, Fv/Fm was significantly affected by light quality of LED lamps. Fv/Fm for scion was the greatest with blue LED, while Fv/Fm for scion significantly decreased under red LED. From these results, it was concluded that variable fluorescence and quantum yield for scion were significantly affected by light quality of artificial lighting source during the healing process of grafted seedlings.

3.2 Graft-taking ratio

Cucumber grafted seedlings graft-taken at PPF of 25 $\mu mol \cdot m^{-2} \cdot s^{-1}$ showed graft-taking ratio higher than 95% and their graft-taking ratios were not significantly affected by different light quality treatment (Table 1). At relatively high PPF of 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$, graft-taking ratio of cucumber seedlings decreased. Among treatments, blue light significantly reduced the graft-taking ratio of grafted seedlings. Cucumber plants acclimated at treatment that blue light was only illuminated or blue light was added showed graft-taking ratio less than 90%.

3.3 Growth characteristics

Leaf area was the greatest under red LED with PPF of 25 $\mu mol \cdot m^{-2} \cdot s^{-1}$, followed by white LED with PPF of 25 $\mu mol \cdot m^{-2} \cdot s^{-1}$, while under blue or white LED with PPF of 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$, leaf area significantly decreased (Table 2). Total fresh weight significantly increased under red illumination with PPF of 25 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Red LED treatment with PPF of 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ showed the highest leaf length. Previously reported studies have demonstrated red light-regulated leaf expansion in plants (Lee et al., 2011; Johkan et al., 2012). However, leaf area, top fresh and dry weight, and SPAD value were significantly inhibited under blue LED with PPF of 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

4 Conclusions

LED lamps are recently introduced to enhance the healing and acclimation of grafted seedlings in some nurseries in Korea. This study was conducted to analyze the chlorophyll fluorescence characteristics of cucumber grafted seedlings healed and acclimated under LED illumination with different light intensity and light quality. Our results demonstrated that the chlorophyll fluorescence characteristics for rootstock and scion of cucumber grafted seedlings were significantly affected by PPF and light quality of LED lamps. Variable fluorescence for scion was the highest with red LED, while variable fluorescence significantly decreased under blue LED during the healing and acclimation of grafted seedlings. Quantum yield for scion was the greatest with blue LED. However, quantum yield for scion significantly decreased with red LED. Cucumber seedlings healed and acclimated under blue light with PPF of 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ showed the lowest graft-taking ratio. Leaf expansion including leaf area and leaf length was significantly decreased with blue LED illumination. From these results, it was concluded that blue light inhibited the graft-taking ratio and growth of cucumber grafted seedlings. And the chlorophyll fluorescence parameters including variable fluorescence and quantum yield can be used to evaluate the joining of scion onto rootstock of grafted seedlings.

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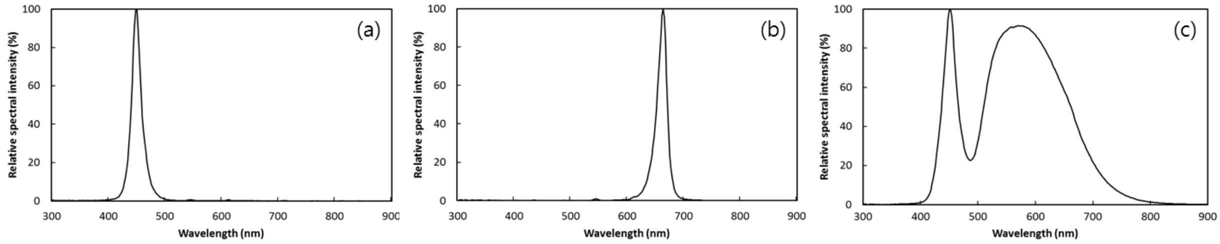

Figure 1: Spectral characteristics of (a) blue, (b) red, and (c) white LED lamps used in this study.

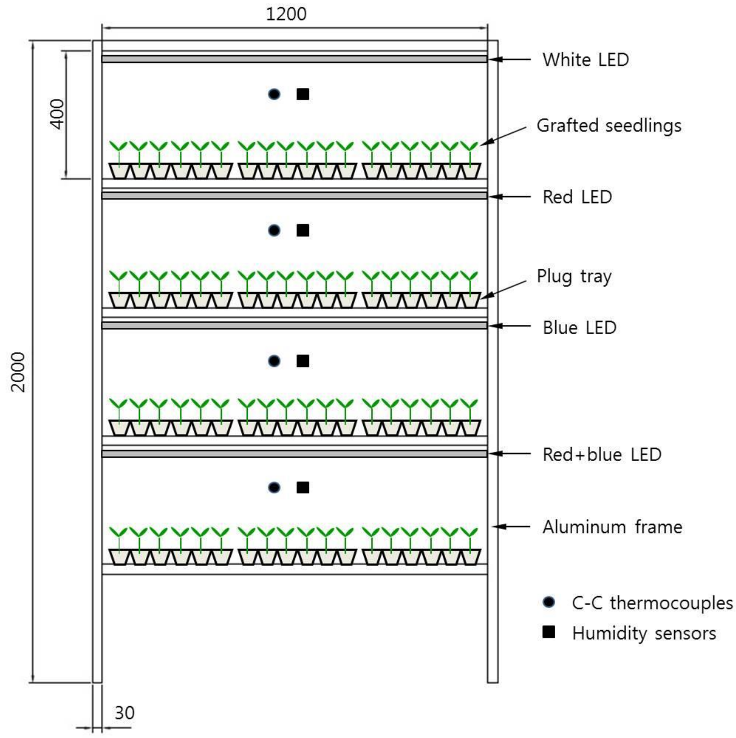

Figure 2: Schematic diagram of a growing bed and grafted seedlings.(unit: mm)

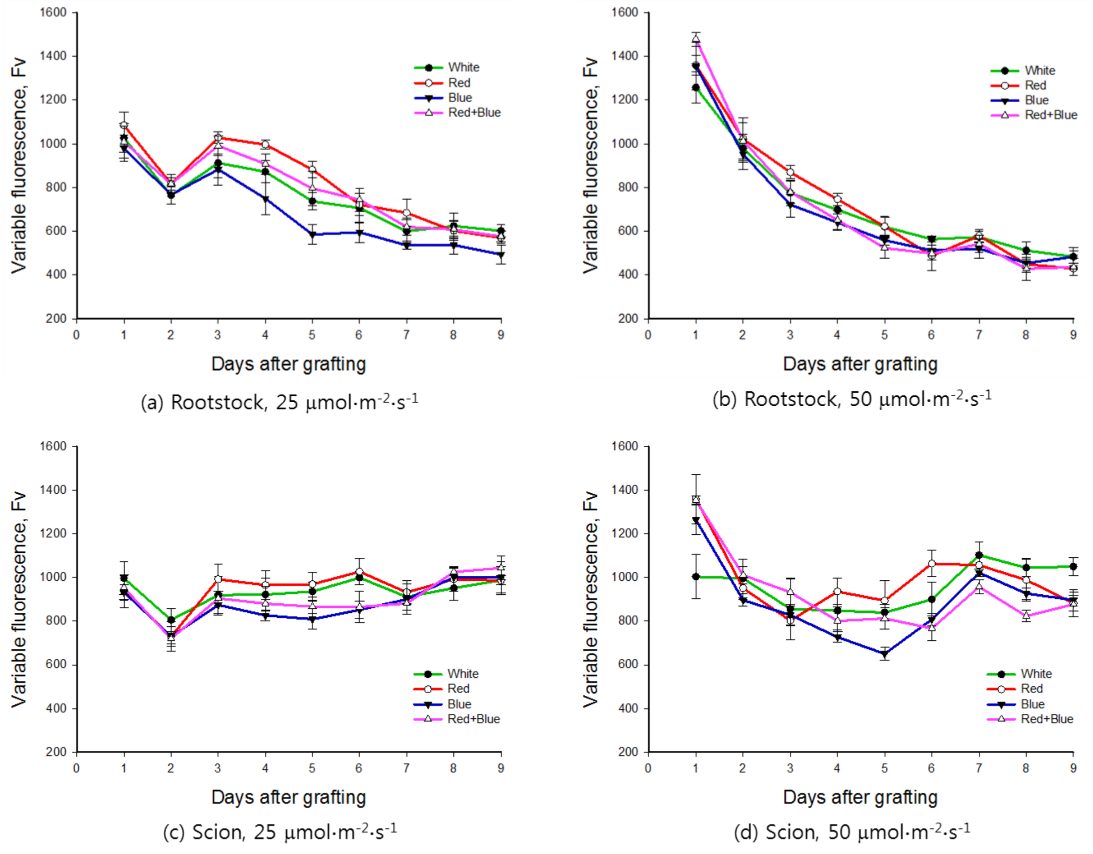

Figure 3: Variable fluorescence of rootstock and scion of cucumber grafted seedlings as affected by photosynthetic photon flux and light quality of LED lamps.

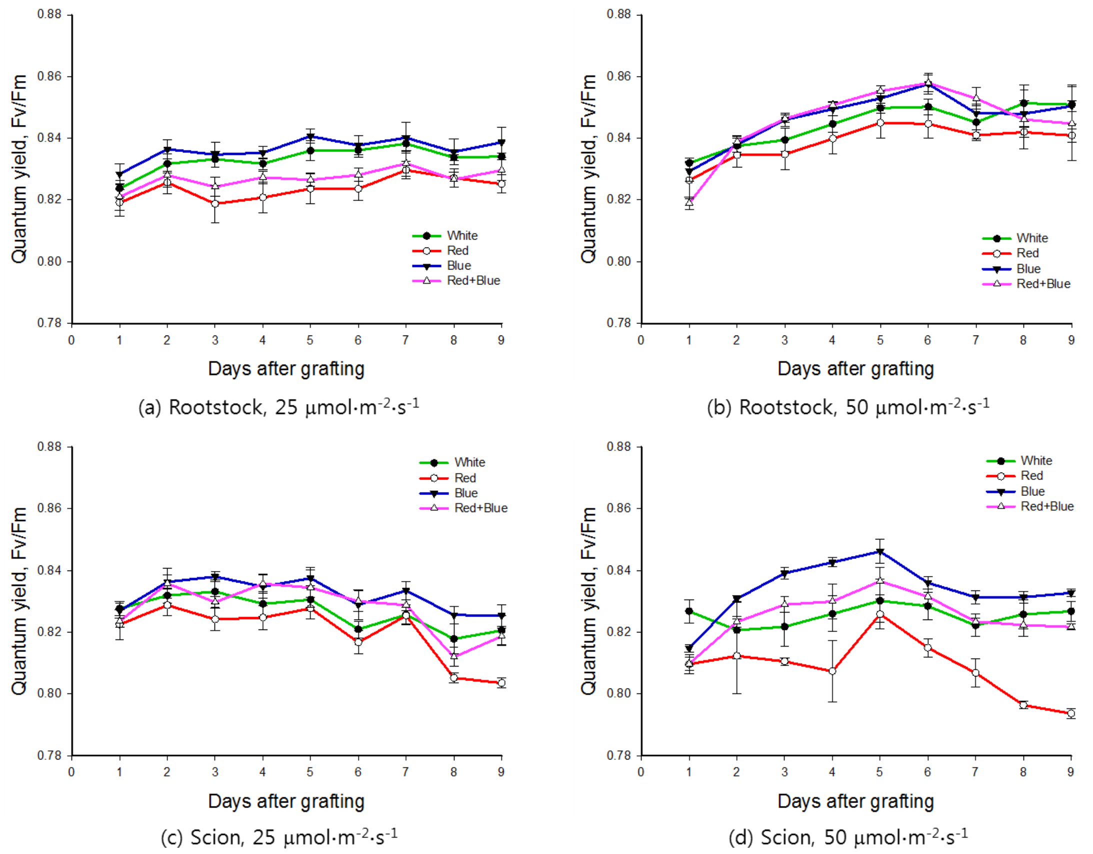

Figure 4: Quantum yield of rootstock and scion of cucumber grafted seedlings as affected by photo-synthetic photon flux and light quality of LED lamps.

Table 1: Graft-taking ratio of cucumber grafted seedlings at 14 days after grafting as affected by photosynthetic photon flux (PPF) and light quality of LED lamps.

PPF ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Light quality	Graft-taking ratio (%)
25	Red	97.5 a[z]
	Blue	95.5 a
	Red+Blue	97.9 a
	White	98.3 a
50	Red	90.0 ab
	Blue	83.3 b
	Red+Blue	88.6 ab
	White	88.3 ab
$LSD_{0.05}$		11.2

[z]Means with the same letter are not significantly different.

Table 2: Growth of cucumber grafted seedlings at 14 days after grafting as affected by photosynthetic photon flux (PPF) and light quality of LED lamps.

PPF ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Light quality	Leaf area (cm^2)	Top fresh weight (g/plant)	Top dry weight (g/plant)	Leaf length (mm)	SPAD value
25	Red	61.71 a[z]	2.278 a	0.201 b	80.1 ab	31.3 c
	Blue	44.68 cd	1.953 bc	0.169 c	74.1 cde	31.2 c
	Red+Blue	42.47 de	1.934 bc	0.230 a	71.0 def	36.5 a
	White	52.17 b	2.004 b	0.217 ab	78.0 bc	35.9 ab
50	Red	48.91 bc	1.879 bcd	0.170 c	82.2 a	35.4 ab
	Blue	35.90 f	1.728 de	0.146 c	70.9 ef	32.2 c
	Red+Blue	37.93 ef	1.818 cde	0.219 ab	67.1 f	35.9 ab
	White	35.69 f	1.677 e	0.156 c	75.0 cd	33.4 bc
$LSD_{0.05}$		5.52	0.198	0.028	4.1	2.6

[z]Means with the same letter are not significantly different.