GGE Biplot as a novel tool for the investigation of marigold (*Tagetes erecta* L.) seedling growth on composted corn stalk as a substrate

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Abstract

This project investigated the feasibility of using ground corn stalks as the substrate to cultivate marigold (*Tagetes erecta* L.). Five treatments including peat moss, composted corn stalks and freshly ground corn stalks were tested for their effects on marigold seedling growth. Seedling quality was described by several morphological and physiological parameters. Data were analyzed using analysis of variance and GGE biplot analysis. There were significant differences among the treatments for several growth parameters, such as seedling biomass, root biomass, stem diameter, leaf area, seedling vigor, chlorophyll content, photosynthetic rate, root activity, stomatal conductance and intercellular CO₂ concentration. Treatment T3, which contains composted ground corn stalks, had the best effect on marigold seedling growth. The results showed that corn stalk was a good substrate for marigold seedlings. GGE biplot demonstrated the substrate effects on marigold seedling quality, and graphically displayed the interrelationships among morphological and physiological parameters. T3 treatment was the best because four morphological parameters, including seedling biomass, roots biomass, stem diameter and seedling vigor, along with six physiological parameters fall into this sector. These results were consistent with the results analyzed by Statistical Analysis Software. For morphological parameters, the correlations are complicated. For physiological parameters, they were all positively correlated between each of two parameters.

Introduction

Marigold (*Tagetes erecta* L.) is one of the most popular herbaceous flowers in the world. It is a commercially important ornamental species as a high-value cut flower or a potted plant for garden settings, landscaping, and roadside planting. In addition, marigold is an important medicinal herb, since its flowers and leaves are often used as food additives. Its unique bright yellow color makes it a favorite plant in landscape design. In recent years, unprecedented urban development has dramatically increased the demand for landscaping and beautification, and has subsequently increased demand for high quality marigold seedlings. In-depth research on marigold has been conducted from different aspects.

Peat moss is commonly used as the substrate for growing marigold seedlings because of its loose nutrient-rich, water retentive and drainage properties. In fact, it has been used as the primary base for most greenhouse and nursery substrates for the last 40 years. However, due to its heavy usage, peat moss resources are decreasing. For physiological parameters, they were all positively correlated between each of two parameters.

Materials and Methods

Yellow marigold *Tagetes erecta* L. var Magic Lady seeds were used. Five soil treatments designated as T1 to T5 were tested: composted corn stalks, fresh ground corn stalks, peat moss, and garden soil (Table 1). The garden soil consisted of field soil, pig manure and chicken manure at a ratio of 6:2:2 (volume). The peat moss was collected from Shuangyang County in Jilin Province. The experiments were conducted in the greenhouse at Jilin Agricultural University from March 2009 to May 2009. Seeds were sown on 5 March, 2009. The seedlings were fertilized once a week with 1% NPK 20-10-20 fertilizer solution. Each soil treatment was tested with 15 trays of plants with 20 seedlings in each tray (57 cm × 28 cm × 5 cm). The experiments were repeated three times. Number of days to first visible flower buds (DFVF) from the time of seed sowing was recorded. All other data concerning morphological and physiological parameters were collected at 40 days after seed sowing. Seedling height and seedling stem diameter were measured using a caliper. Seedling biomass was the dry weight of the whole plant measured after oven drying at 70°C overnight. Photosynthetic rate, stomatal conductance, transpiration rate, and intercellular CO₂ concentration were measured using an American CI-340 portable photosynthesis system. Root activity was

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measured using the triphenyltetrazolium chloride method. Leaf area was measured using an American CI-203 laser. Chlorophyll content was determined using an acetone extraction spectrophotometer. Data were analyzed by a two-way analysis of variance with treatments (SAS 9.2, Cary, USA). The means of each parameter were compared using Duncan’s multiple range tests. \( P=0.05 \) was considered significant. Two-way data were further analyzed and displayed using GGE-biplot system. The GGE biplot method was employed to study the genotype by site interaction of yield based on the formula:

\[
Y_{ij} = \mu + \alpha_i + \beta_j + \phi_{ij}
\]

In this experiment, \( Y_{ij} \) is the expected value for the parameters in the \( j \) (environment), \( \mu \) is the mean values in the \( j \) (environment), \( \alpha_i \) is the effects of growth parameters such as biomass, roots biomass, stem diameter, etc., \( \beta_j \) is the effect of the treatments, \( \phi_{ij} \) is the interaction between the observed value of these parameters and the treatments. To generate a GGE biplot, all data have been transferred by a scaling and data-centering method. After scaling, the first principal component (PC1) and second principal component (PC2) have the same unit. A biplot is constructed by plotting the PC1 scores against the PC2 scores for each treatment.

**Results and Discussion**

As shown in Table 2, there were significant differences among treatments regarding the effects on all marigold seedling growth parameters. The T3 treatment produced the greatest difference compared to other treatments. Seedlings in T3 treatment were the best for the five growth parameters of seedling biomass, root biomass, stem diameter, leaf area, and seedling vigor.

GGE biplot was helpful to analyze the relationships between the morphological growth parameters and treatments. The effects of PC1 score and PC2 score were 83.2% and 14.1%, respectively. The sum effect of the principal component was 97.3%. This means the GGE-biplot explained 97.3% of the total variation of the data. Therefore, the performance of marigold seedlings under different treatments can be compared on the GGE biplot. Seven morphological growth parameters were distributed into five sectors (Figure 1). Four growth parameters fall into T3 sector, including seedling biomass, roots biomass, stem diameter and seedling vigor, indicating that T3 treatment was the best among these treatments.

### Table 1. Substrate and its components for marigold seedling growth.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Components (v/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>Composted ground corn stalks: garden soil* 1:2</td>
</tr>
<tr>
<td>T2</td>
<td>Ground corn stalks: garden soil* 1:2</td>
</tr>
<tr>
<td>T3</td>
<td>Composted ground corn stalks: garden soil* 1:3</td>
</tr>
<tr>
<td>T4</td>
<td>Ground corn stalks: garden soil* 1:3</td>
</tr>
<tr>
<td>T5 (Control)</td>
<td>Peatmoss: garden soil* 1:3</td>
</tr>
</tbody>
</table>


### Table 2. Different treatments effect the morphological growth parameters of marigold seedling.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Seedling biomass (mg)</th>
<th>Roots biomass (mg)</th>
<th>Stem diameter (cm)</th>
<th>Plant height (cm)</th>
<th>Left area (cm²)</th>
<th>DFV*</th>
<th>Seedling vigor**</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>122.74</td>
<td>27.38</td>
<td>0.25</td>
<td>7.35</td>
<td>11.88</td>
<td>68</td>
<td>39.41</td>
</tr>
<tr>
<td>T2</td>
<td>101.21</td>
<td>26.63</td>
<td>0.22</td>
<td>8.23</td>
<td>9.86</td>
<td>72</td>
<td>38.84</td>
</tr>
<tr>
<td>T3</td>
<td>150.76</td>
<td>38.36</td>
<td>0.27</td>
<td>6.73</td>
<td>12.01</td>
<td>66</td>
<td>57.49</td>
</tr>
<tr>
<td>T4</td>
<td>136.83</td>
<td>32.53</td>
<td>0.24</td>
<td>7.31</td>
<td>10.02</td>
<td>72</td>
<td>47.17</td>
</tr>
<tr>
<td>T5</td>
<td>142.69</td>
<td>34.34</td>
<td>0.26</td>
<td>6.71</td>
<td>12.25</td>
<td>66</td>
<td>50.75</td>
</tr>
</tbody>
</table>

*Mean separation within columns by least significant difference. \( P=0.05 \).
**Numbers of days to first visible flower buds from time when sown.

**Seedling vigor = \( \text{stem diameter (cm) / seedling height (cm) + roots biomass (g) / aboveground portion biomass (g)} \times \text{whole seedling biomass (g).} \)

![Figure 1. Different treatments affect the morphological growth parameters of marigold seedling. A, seedling biomass; B, root biomass; C, stem diameter; D, plant height; E, leaf area; F, days taken to start blooming; G, seedling vigor.](image1)

![Figure 2. Correlations among different treatments and their effects on morphological growth parameters of marigold seedling. A, seedling biomass; B, root biomass; C, stem diameter; D, plant height; E, leaf area; F, days taken to start blooming; G, seedling vigor.](image2)
five treatments. Two parameters, plant height and number of DFVF, were distributed in T2 sector indicated that T2 treatment was good, but not as good as T3. For a flowering plant, high plant height and high DFVF mean that excessive vegetative growth occurred due to some unfavorable factors. One parameter, leaf area, was distributed in T5 sector. It means that T5 treatment favors increased leaf area. This interpretation was consistent with the result of analysis by Duncan’s test.

The interaction and correlations among various parameters can also be analyzed using GGE biplot. In the biplot, the connection line from parameter points to the coordinate origin is called the target vector. When considering the target vector as a starting point, then rotating it clockwise, the cosine of the angle between the vectors approximates the correlation coefficients between them. A 90° angle means a zero correlation (completely independent), a 0° angle means a correlation of +1, and a 180° angle means a correlation of -1. An acute angle indicates a positive correlation, while an obtuse angle indicates a negative correlation. The most prominent relations revealed by the biplot (Figure 2) are: i) leaf area was positively correlated with stem diameter, seedling biomass, roots biomass and seedling vigor; ii) leaf area was negatively correlated with plant height and DFVF; and iii) root biomass and seedling vigor were the most closely correlated, with a correlation coefficient of 0.9984. This indicates that seedling vigor was affected by root biomass. The higher value of root biomass indicates that roots developed well, which promoted the growth of shoots, thereby raising the parameters of seedling vigor; iv) leaf area was negatively correlated to plant height with a correlation coefficient of -0.7999. An increased leaf area leads to a lower plant height, which is beneficial for seedling vigor; v) leaf area and DFVF were negatively correlated with a correlation coefficient of -0.9793. This indicates that a bigger leaf area can promote flower bud differentiation. A possible reason was an increased leaf area promoting photosynthesis, thus accelerating the growth of marigold seedlings; vi) stem diameter and plant height were negatively correlated, with a correlation coefficient of -0.9576. This means inhibiting plant shoot elongation can increase the stem diameter and improve seedling quality. But increased stem diameter delayed blooming time, which may be due to competition for nutrition. Excessive vegetative growth delayed reproductive growth; and vii) leaf area and DFVF were negatively correlated to plant height and DFVF. This provided evidence that there were complicated relationships and interactions among different growth parameters. Reasonable adjustments of nursery conditions were necessary according to the status of seedling growth. In our experiments, we found T3 treatment was the best for marigold seedling growth. As shown in Table 3, all physiological parameters except for transpiration rate improved in T3 treatments were also beneficial to root biomass development. This conclusion was consistent with that concerning root biomass described above.

Figure 3 shows the effects of substrates on the physiological growth parameters of marigold seedlings by GGE biplot. This figure explained 90.3% (sum PC) of the total variation of the data. In Figure 3, six physiological parameters, except for transpiration rate, were assigned to the T3 sector. These results show that the T3 treatment was the most favorable for improving physiological function and promoting the growth of marigold seedlings. Interrelationships among different physiological parameters are shown in Figure 4. There was a positive correlation among all physiological parameters between each of two parameters, such as between chlorophyll content and root activity, between stomatal conductance and intercellular CO2 concentration; correlation coefficients were 0.8805 and 0.8130, respectively. This means that an improvement in root activity could promote uptake and transportation of nutrients, and then increase the chlorophyll content in the leaves, thus promoting photosynthesis and seedling growth. Similarly, the improvement of stomatal conductance had beneficial effects on the concentration of intercellular CO2 and, therefore, improved the efficiency of photosynthesis, and subse-
Table 3. Different treatments effect the physiological growth parameters of marigold seedlings.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Chlorophyll content (mg×dm⁻²)</th>
<th>Photosynthetic rate (umolCO₂·m⁻²·s⁻¹)</th>
<th>Root activity (mg·g⁻¹·h⁻¹)</th>
<th>Stomatal conductance (mol water·m⁻²·s⁻¹)</th>
<th>Transpiration rate (mmol·cm⁻²·s⁻¹)</th>
<th>Intercellular CO₂ concentration (umol/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>2.01</td>
<td>1.51</td>
<td>1.72</td>
<td>0.21</td>
<td>2.48</td>
<td>221</td>
</tr>
<tr>
<td>T2</td>
<td>1.84</td>
<td>1.38</td>
<td>1.12</td>
<td>0.14</td>
<td>1.73</td>
<td>202</td>
</tr>
<tr>
<td>T3</td>
<td>2.25</td>
<td>1.91</td>
<td>1.32</td>
<td>0.23</td>
<td>2.24</td>
<td>248</td>
</tr>
<tr>
<td>T4</td>
<td>1.95</td>
<td>1.78</td>
<td>1.21</td>
<td>0.15</td>
<td>1.97</td>
<td>212</td>
</tr>
<tr>
<td>T5</td>
<td>2.02</td>
<td>1.87</td>
<td>1.29</td>
<td>0.19</td>
<td>2.12</td>
<td>206</td>
</tr>
</tbody>
</table>

Mean separation within columns by least significant difference, P=0.05.

Consecutively promoted seedling growth.

Seedling quality was described by several morphological and physiological parameters. There were significant differences in growth parameters among treatments, including seedling biomass, root biomass, stem diameter, leaf area, seedling vigor, chlorophyll content, photosynthetic rate, root activity, stomatal conductance, and intercellular CO₂ concentration. T3 treatment (compost of 1:3 corn stalks: garden soil) was the best for marigold seedling growth among the five treatments tested.

Results revealed by GGE biplot demonstrated that T3 treatment was the best because four morphological parameters, including seedling biomass, root biomass, stem diameter, and seedling vigor, and six physiological parameters fall into this sector. These results were consistent with the results analyzed by Statistical Analysis Software method (Duncan’s test). Our research suggests that composted corn stalk may be a good substrate for growing marigold and other ornamental plants. Using corn stalks instead of peat moss as a nursery substrate may be beneficial because corn stalks are a nutrient-rich, low cost, and renewable resource, and thus could help alleviate the depletion of peat moss resources. This study was the first of its kind to use the GGE biplot to analyze and interpret substrate effects on marigold seedlings. This analytical technique provided a means of not only evaluating the effects of different substrate components on the physiological and morphological growth parameters of flower seedlings, but also provided a summary of the relationships among the different parameters assessed.

Conclusions

Despite their apparent potential, there are limitations to using corn stalks as a base for growing seedlings. Some investigations reported that corn stalk compost had some negative effects because it produced water and nitrogen stress on seedling cultivation, especially during the initial growing stage. This water and nitrogen stress phenomenon was not observed in our research. The reason was probably due to the improvement we made by mixing the composted corn stalk with other substrates, such as pig or chicken manure, to provide the required nutrients for the seedlings.

References