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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 CO_2 concentration. Treatment T3, which contained 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.[1] 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.[2-8]

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 dwindling and their replenishment requires time and conservation. Therefore, there is a need to identify an alternate substrate that is of consistent quality, is renewable, economically priced and readily available. Alternative substrates or substrate amendments have been investigated and ground pine chips seem to be a good candidate for container substrates for greenhouse and nursery crops.[9-15] However, other materials might also provide good substrates. For example, recently Zhang *et al.*[16] reported positive results using corn stalks to grow vegetable seedlings. In their study, they found corn stalk mixed with garden soil at a ratio of 2:3 to be a good substrate for growing pepper seedlings. Based on the preliminary findings of Zhang *et al.*,[16] the purpose of this study was to investigate the feasibility of using corn stalks as a substrate to grow marigold seedlings. The objective of our research was to compare the ability of composted cornstalks to produce marigold seedling growth with seedlings grown in peatmoss.

Another purpose of the study was to apply the GGE biplot method to analyze the effects of different substrates on marigold seedling growth. The GGE biplot methodology was developed for graphical analysis of genotype by environment data or multi-environment trial data. It was originally developed to help plant breeders to understand and explore genotype by environment interactions.[17-22] This research was the first study beyond genetics and breeding to apply GGE biplot to analyze data concerning morphological and physiological characteristics of marigold seedlings. Our research aimed to explore the feasibility of using renewable corn stalks as a substrate replacement for peat moss to grow an economically important ornamental plant species. The use of the GGE biplot can not only provide multiple range comparisons among results by conventional analytical method, but also demonstrate the correlations and interactions among parameters in one graph that is intuitive and easy to understand.

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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 CO_2 concentration were measured using an American CI-340 portable photosynthesis system. Root activity was measured using the triphenyltetrazolium chloride method.[23-24] Leaf area was measured using an American CI-203 laser. Chlorophyll content was determined using an acetone extraction spectrophotometer.[25] 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: [17]

$$Y_{ij}=\mu+\alpha_i+\beta_j+\phi_{ij}$$

In this experiment, Y_{ij} is the expected value for the parameters in the j (environment), μ is the mean values in the j (environment), α_i is the effects of growth parameters such as biomass, roots biomass, stem diameter, etc., β_j is the effect of the treatments, ϕ_{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

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

Treatment	Components (v/v)	
T1	Composted ground corn stalks: garden soil*	1:2
T2	Ground corn stalks: garden soil*	1:2
T3	Composted ground corn stalks: garden soil*	1:3
T4	Ground corn stalks: garden soil*	1:3
T5 (Control)	Peatmoss: garden soil*	1:3

*Garden soil contains field soil: pig manure: chicken manure = 6:2:2 (v/v).

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

Treatment	Seedling biomass (mg)	Roots biomass (mg)	Stem diameter (cm)	Plant height (cm)	Left area (cm^2)	DFVF*	Seedling vigor**
T1	122.74	27.38	0.25	7.35	11.88	68	39.41
T2	101.21	26.63	0.22	8.23	9.86	72	38.84
T3	150.76	38.36	0.27	6.73	12.01	66	57.49
T4	136.83	32.53	0.24	7.31	10.02	72	47.17
T5	142.69	34.34	0.26	6.71	12.25	66	50.75

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 = [stem diameter (cm) / seedling height (cm) + roots biomass (g) / aboveground portion biomass (g)] x whole seedling biomass (g).

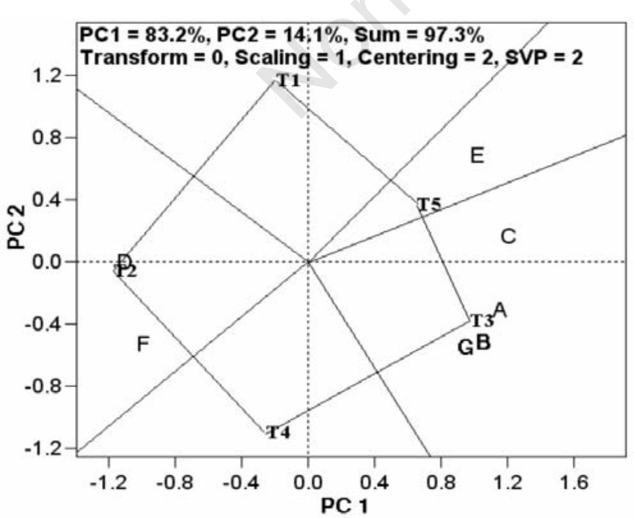

Figure 1. Different treatments affect the morphological growth parameters of marigold seedling. A, seedling biomass; B, root biomass; C, stem diameter; D, plant height; E, leaf area; F, days taken to start blooming; G, seedling vigor.

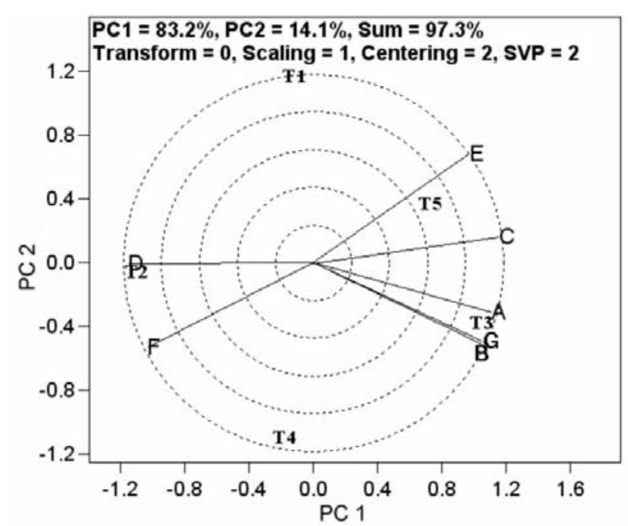

Figure 2. Correlations among different treatments and their effects on morphological growth parameters of marigold seedling. A, seedling biomass; B, root biomass; C, stem diameter; D, plant height; E, leaf area; F, days taken to start blooming; G, seedling vigor.

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 section. 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.[21,26] 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) seedling biomass, seedling vigor and root biomass were positively 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 reached the highest values in T3 treatments. This indicates the substrates in T3 treatments increased chlorophyll content and, therefore, improved photosynthesis. The root activity of marigold seedlings in the T3 treatments was also significantly higher than in other treatments. Substrates in T3 treatments were also beneficial to root 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 CO_2 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 CO_2 and, therefore, improved the efficiency of photosynthesis, and subse-

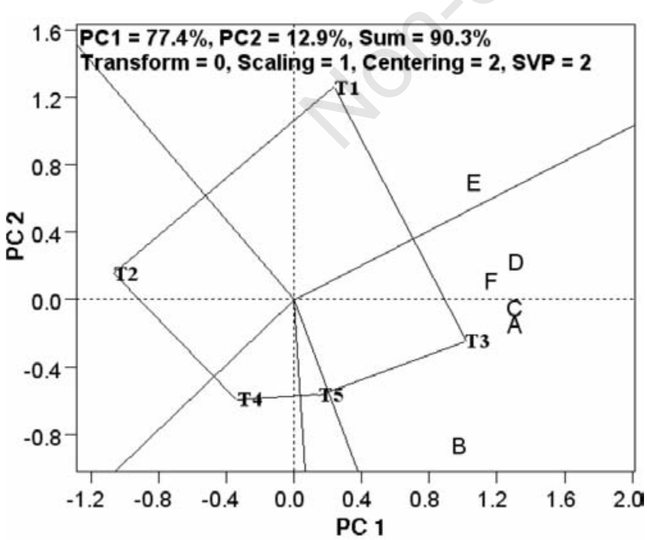

Figure 3. Different treatments affect the physiological growth parameters of marigold seedling. A, chlorophyll content; B, photosynthetic rate; C, root activity; D, stomatal conductance; E, transpiration rate; F, intercellular CO_2 concentration.

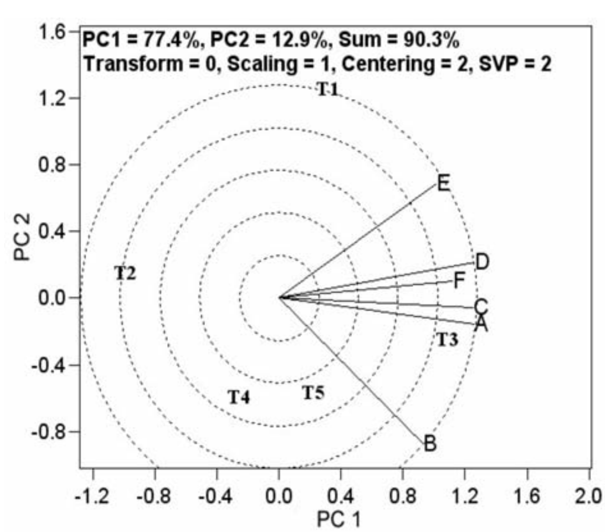

Figure 4. Correlations among different treatments and their effects on physiological growth parameters of marigold seedling. A, chlorophyll content; B, photosynthetic rate; C, root activity; D, stomatal conductance; E, transpiration rate; F, intercellular CO_2 concentration.

Table 3. Different treatments effect the physiological growth parameters of marigold seedlings.

Treatment	Clorophyll content ($mg \times dm^{-2}$)	Photosynthetic rate ($umolCO_2 \times m^{-2} \times s^{-1}$)	Root activity ($mg \times g^{-1} \times h^{-1}$)	Stomatal conductance (mol water$\times m^{-2} \times s^{-1}$)	Transpiration rate ($mmol \times m^{-2} \times s^{-1}$)	Intercellular CO_2 concentration (umol/mol)
T1	2.01	1.51	1.27	0.21	2.48	221
T2	1.84	1.36	1.12	0.14	1.73	202
T3	2.25	1.91	1.32	0.23	2.24	248
T4	1.95	1.78	1.21	0.15	1.97	212
T5	2.02	1.87	1.29	0.19	2.12	206

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

quently 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 CO_2 concentration. T3 treatment (compost of 1:3 corn stalks: garden soil) was the best for marigold seeding 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.[16] 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

1. Zhang J, Xu J, Li F, Shen Y. A review of studies on African marigold (Tagetes erecta L.). Chinese Southwest Hort 2005;33:17-20.
2. Atiyeh RM, Edwards CA, Subler S, et al. Earthworm-processed organic wastes as components of horticultural potting media for growing marigold and vegetable seedlings. Compoist Sci 2000;8:215-23.
3. Wan Q, Hu Z. Study of liquid medium solution composition for Marigold. J Seed 2002;1:37-8.
4. Linderman RG, Davis EA. Varied response of marigold (Tagetes spp.) genotypes to inoculation with different arbuscular mycorrhizal fungi. Sci Hort 2004;99:67-78.
5. Burnett SE, Ierseland Van MW, Thomas PA. Medium-incorporated PEG-8000 reduces elongation, growth, and whole canopy carbon dioxide exchange of marigold. Hort Sci 2006;41:124-30.
6. Magnitskiy SV, Pasian CC, Bennett MA, et al. Controlling height and seedling emergence of French marigold and celosia plugs with plant growth regulators. J Envir Hort 2006;24:165-8.
7. Van Iersel MW, Seymour L. Temperature effects on photosynthesis, growth respiration, and maintenance respiration of marigold. Acta Hort 2003;624:549-54.
8. Van Iersel MW, Gardestrom P. Respiratory Q10 of marigold (Tagetes patula) in response to long-term temperature differences and its relationship to growth and maintenance respiration. Physi Planta 2006;128:289-301.
9. Chong C, Cline RA. Response of four ornamental shrubs to container substrate amended with two sources of raw paper mill sludge. Hort Sci 1993;28:807-9.
10. Atiyeh RM, Edwardsa CA, Sublerb S, et al. Pig manure vermicompost as a component of a horticultural bedding plant medium: effects on physicochemical properties and plant growth. Biores Technol 2001;78:11-20.
11. Heo J, Lee C, Chakrabarty D, et al. Growth responses of marigold and salvia bedding plants as affected by monochromic or mixture radiation provided by a Light-Emitting Diode (LED). Plant Growth Regul 2002;38: 225-30.
12. Wright RD, Browder JF. Chipped pine logs: a potential substrate for greenhouse and nursery crops. Hort Sci 2005;40:1513-5.
13. Wright RD, Browder JF, Jackson BE. Ground pine chips as a substrate for container-grown woody nursery crops. J Environ Hort 2006;24:181-4.
14. Chen JJ, Li YC. Coal fly ash as an amendment to container substrate for Spathiphyllum production. Biores technol 2006;97:1920-6.
15. Bachman GR, Metzger JD. Growth of bedding plants in commercial potting substrate amended with vermicompost. Bioresource Technology 2008;99:3155-61.
16. Zhang X, Zhang X, Sun K. The application of corn stalk on sweet pepper seedling. Chinese Northern Hort 2007;9:20-2.
17. Yan W, Cornelius PL, Crossa J, et al. Two types of GGE biplots for analyzing multi-environment trial data. Crop Sci 2001;41: 656-63.
18. Yan W. GGE biplot - a Windows application for graphical analysis of multi-environment trial data and other types of two-way data. Agron J 2001;93:1111-8.
19. Yan W, Hunt LA, Sheng Q, et al. Cultivar evaluation and mega-environment investigation based on GGE biplot. Crop Sci

2000;40:597-605.
20. Yan W, Sheng Q, Hu YG, et al. GGE biplot - an ideal method for graphical analysis of genotype by environment interaction. Acta Agro Sinica 2001;27:21-8.
21. Yan W, Tinker NA. An integrated system of biplot analysis for displaying, interpreting, and exploring genotype by environment interactions. Crop Sci 2005;45:1004-16.
22. Shang Y, Li SQ, Li DR, et al. GGE biplot analysis of a backcross population of B. napus L. Acta Agro Sinica 2006;32:243-8.
23. Joslin JD, Henderson GS. The determination of percentages of living tissue in woody fine root samples using triphenyltetrazolium chloride. Forest Sci 1984;30: 965-70.
24. Clemensson-Lindell A. Triphenyltetrazolium chloride as an indicator of fine-root vitality and environmental stress in coniferous forest stands: applications and limitations. Plant and Soil 1994;159:297-300.
25. Lichtenthaler HK, Wellburn AR. Determination of total carotenoids and chlorophylls A and B or leaf in dissolved solvents. Biol Soc Trans 1985;11:591-2.
26. Yan W, Rajcan I. Biplot evaluation of test sites and trait relations of soybean in Ontario. Crop Sci 2002;42:11-20.