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Evaluation of the Effects of Grafting and Vermicompost Applications on the Morphophysiological Properties of Eggplant under Drought Stress with Principal Component Analysis

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ABSTRACT

Drought is one of the important abiotic stresses that significantly affect plant growth and development process. The use of grafted plants and vermicompost application creates significant potential for tolerance to drought stress. The scope of the study; consists of the evaluation of the effects of grafting and vermicompost applications on the morphophysiological properties of eggplant under drought stress by principal component analysis (PCA). In the experiment, different amounts of vermicompost-V (0, 1, 2, 3%) were applied to the grafting and non-grafting plants under different levels of drought stress conditions (Control; 100%; mild stress-MS; 70% and severe stress-SS; 30% irrigation) at the greenhouse. In terms of traits studied, the first two of the components had 94.39% of the total variation in the grafted plants. PCA showed that 'V(3%)+MS' and 'V(2%)+MS' were in significant and positive correlations with SPAD, leaf area-LA, relative water content-RWC, shoot dry weight-SDW, and root fresh weight -RFW. 'V(%3)+MS' and 'V(%2)+MS' applications; shoot length-SL correlated positively and weakly with stomata conductivity-g_s, shoot fresh weight-SFW, shoot diameter-SD, and root dry weight-RDW. Based on the relationships between the variables; in general, the correlations of all the examined parameters with each other were found to be significant and positive. Especially; the positive and significant correlations between SPAD and LA and RWC, SDW and RFW, g_s and SL and SFW, and RDW and SD were obvious. Consequently, the use of grafted plants and V treatments in eggplants under MS conditions improved the morphophysiological parameters of the plants and increased their tolerance to stress. Therefore, it can be seen as an effective strategy for sustainable agricultural practices.

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Keywords
Solanum melongena L.
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Aşılama ve Vermikompost Uygulamalarının Kuraklık Stresi Altındaki Patlıcanın Morfofizyolojik Özellikleri Üzerine Etkilerinin Temel Bileşen Analizi ile Değerlendirilmesi

ÖZET

Kuraklık, bitki büyümesini ve gelişim sürecini önemli ölçüde etkileyen önemli abiyotik streslerden biridir. Aşılı bitki kullanımı ve vermikompost uygulaması kuraklık stresine tolerans sağlama bakımından önemli potansiyel oluşturmaktadır. Çalışmanın amacı; aşılı bitki kullanımı ve vermikompost uygulamalarının kuraklık stresi altında patlıcanın morfofizyolojik özellikleri üzerine etkilerinin temel bileşen analizi (PCA) ile değerlendirilmesidir. Sera koşullarında gerçekleştirilen çalışmada aşılı ve aşısız bitkiler kuraklık stresi altında farklı seviyelerde (%0, 1, 2, 3) solucan humusu-V içeren koşullarda yetiştirilmiştir (kontrol: %100, hafif stres-MS: %70 ve şiddetli stres-SS: %30 sulama). İncelenen

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özellikler açısından, bileşenlerin ilk ikisi aşılanmış bitkilerdeki toplam varyasyonun %94.39'unu oluşturmaktadır. PCA, 'V(%3)+MS' ve 'V(%2)+MS'nin, SPAD, yaprak alanı-LA, bağıl su içeriği-RWC, sürgün kuru ağırlığı- SDW ve kök kuru ağırlığı-RFW ile anlamlı ve pozitif korelasyon içinde olduğunu göstermiştir. 'V(%3)+MS' ve 'V(%2)+MS' uygulamaları; sürgün uzunluğu-SL, stoma iletkenliği-g_s, sürgün yaş ağırlığı-SFW, gövde kalınlığı- SD ve kök kuru ağırlığı-RDW ile pozitif ve zayıf korelasyon sergilemiştir. Genel olarak incelenen tüm özelliklerin birbirleriyle ilişkilendirilmeleri, anlamlı ve pozitif bulunmuştur. Özellikle SPAD, LA ve RWC; SDW ve RFW; g_s, SL ve SFW arasındaki ve RDW ile SD arasındaki pozitif ve güçlü korelasyonlar dikkat çekici olmuştur. Sonuç olarak patlıcanlarda aşılı bitki kullanımı ve hafif stres koşullarında vermikompost uygulamaları, bitkilerin morfofizyolojik özelliklerini olumlu yönde etkilemiş ve strese karşı toleranslarını artırmıştır. Bu nedenle sürdürülebilir tarım uygulamaları için etkili bir strateji olarak görülebilir.

Vermikompost
Abiotik stress
PCA

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INTRODUCTION

Drought stress; it occurs when the precipitation amount is insufficient and evaporation is more than water intake (Rao et al., 2006). Plant growth slows when soil moisture decreases, and as drought stress increases, crop yield quality suffers, eventually leading to plant mortality (Ashraf & Harris 2005). Water starvation in plant cells results in decreased soluble nutrient density, decreased turgor pressure, changes in cell volume, and degeneration of the structures of several physiological and molecular components in proteins (Wahab et al., *2022).* Morphological and physiological changes such as a reduction in leaf area, a decrease in stomata number, relative water content, stomatal conductivity and a decrease in chlorophyll content occur in many vegetable species under drought stress (Ors et al., 2021; Sousa et al., 2022; Wassie et al., 2023).

After potatoes and tomatoes, eggplant is the third most significant vegetable in the Solanaceae family in terms of production (Sekara et al., 2007). Although eggplant is a moderately drought-tolerant vegetable, it is known that limited water applications can cause drought stress, metabolic activities can be impaired in case of prolonged drought, plant growth and yield are adversely affected, and even irreversible plant damage occurs (Jifeng et al., 2009; Kurniawati 2014). The use of tolerant varieties is seen as a permanent solution for reducing yield and quality losses in drought stress conditions for economically important cultural plants like eggplant. Tolerance to drought stress is controlled by many genes which complicate breeding efforts to develop drought-tolerant varieties (Toppino et al., 2022; Villanueva et al., 2023). However, there are effective strategies to reduce the negative effects of drought stress on plants, such as grafting on rootstocks with high drought resistance and the use of organic materials such as vermicompost (V) (Boyaci & Ellialtioglu, 2018; Ebrahimi et al., 2021).

It is crucial to graft on rootstocks with a high ability to withstand drought conditions and increase water use efficiency (WUE) to reduce production losses. It was determined that the use of grafted plants on rootstocks with high resistance to stress in eggplant under limited water conditions gave positive results on crop yield and quality and it was seen that it gave positive results as a curative application (Kıran et al., 2019a). However, in cases where the severity of drought stress is high this application alone may be insufficient. In this situation, the usage of organic materials such as that support to increase in the water-holding capacity of the soil can be effective in plant drought tolerance (Kıran, 2019b; Istanbuli et al., 2020).

Drought stress is a multi-faceted stress factor that directly affects plant growth and development physiology, As well as the plant the plant cell organ, and the whole organism. Conventional methods for statistically evaluating a vast amount of data relating to morphological, physiological, and biochemical characteristics in order to understand plant responses to stress conditions involve useful knowledge for each variable studied. However, these methods may be insufficient to explain the state of the relationship between two or more features and to make real interpretations based on this. Therefore, a multivariate statistical analysis method such as Principal Component Analysis (PCA) is needed in order to make the variables easier to interpret.

In the completed study, the effects of grafted plant and vermicompost applications on the development of eggplants grown under drought stress conditions were examined in terms of morphological and physiological characteristics, and the positive effect of the grafting and vermicompost interaction on stress tolerance was determined (Kıran et al., 2023). In this study, the effect of the positive interaction of grafting and vermicompost on some morphological and physiological characteristics of eggplant grown under drought stress was evaluated by principal component analysis (PCA).

MATERIALS and METHOD

The study was conducted between April and July 2020, under naturally lighted glasshouse conditions at the Ankara-Soil, Fertilizer and Water Resources Central Research Institute (Ankara, Türkiye). The temperature and humidity control was provided automatically (23-25 ^{o}C temperature. 50% to 55% relative humidity). Local variety Aydın Siyahı, having long shaped and black colored fruits, as scion source and a commercial rootstock Köksal F_1 (Yüksel Seeds Co., Türkiye) were used as plant material. The grafting process was carried out in a private nursery Antalya Tarım Co., Türkiye). The seedlings at the 3-4 true leaf stage were planted in polyethylene pots with a size of 39×35 cm and a volume of 35 L containing clay texture soil (organic matter: 0.51% available phosphorus: 16.7 kg ha^{-1} available potassium: 546.7 kg ha^{-1}, EC: 0.94 dS m^{-1}, pH: 7.90). Fifteen days before planting V was weighed at 1%, 2% and 3% (w/w) of the soil weight in the pot and mixed to a depth of 10-15 cm.

Vermicompost (V) (organic matter: 39.2%, total phosphorus: 0.21%, total potassium: 0.94%, EC:5.62 dS m-1, pH:6.62) was obtained from Ekosol Farming & Livestock Company, Manisa, Türkiye. Stress applications were initiated 10 days after planting the seedlings. The experiment was conducted in random plots with three replications per treatment with five plants per replication adopting the factorial (Factor 1: grafted plant, Factor 2: V application, Factor 3: drought stress) experimental. Accordingly, the subjects were as follows: 1. irrigation level-drought stress: bringing the current moisture to the field capacity (Control: 100%), applying 70% (mild stress-MS) and 30% (severe stress-SS) of the water, 2. grafting (G), usage of non-grafted plant, use of the grafted plant, 3. V application at levels of 0 (control), 1%, 2%, 3%. According to the results of soil analysis, each pot received 10 kg of phosphorus and 7 kg of nitrogen per 0.247 acre in the form of triple superphosphate (TSP) and urea before planting. During the flowering and the beginning of harvest, urea fertilization was continued in each pot in the form of 3 kg of nitrogen per 0.247 acre.

Measurements of plant growth

Shoot (stem+leaves)fresh weight (SFW) and dry weight (SDW) (g plant^{-1}), At the end of the production period the fresh weight of the shoot and leaves were weighed on a 1 1000-1 precision digital scale to measure the green part's wet weight (g) and after drying in an oven set at 65^{o}C the green part dry weight (g) was detected. Shoot length (cm) (SL), At the end of the growing period, the shoot height of the plants in all application groups from the root collar to the growth tip of the plant was measured in cm using a tape measure. The shoot diameter (SD) of the plants in all treatment groups was measured using a digital caliper. The mean shoot thickness (mm) value was obtained by averaging the shoot thickness measurements taken from a point just above the graft site half the plant height and the uppermost tip node. Leaf area (cm^2) (LA) at the date of the second fruit harvest in the growing period the 3rd and 4th leaves of each plant were taken backward from the shoot tip and measured with the Licor LI-3000A (Li-Cor Lincoln, USA) model leaf area meter and the 'leaf area/plant' value was obtained.

Measurements related to the physiological properties of the plant

Leaf chlorophyll content was measured using a SPAD chlorophyll meter (Minolta, SPAD 502, Osaka, Japan). SPAD values of the uppermost fully expanded leaves were determined by reading the 3rd leaf from the top of each plant. Stomatal conductance (g_s) with a handheld steady-state portable porometer (SC-1, Decagon Devices Inc., Pullman, WA, USA) was used to measure stomatal conductivity. It was obtained by measuring between 13.00-14.00 with a fifteen-day interval on the same leaves that were randomly selected during the experiment. Leaf proportional water content (RWC) was measured using the method described by Dhanda & Sethi (1988).

Data analysis

Principal component analysis (PCA) was applied with the MS Excel XLSTAT program to determine the relationships between the investigated parameters.

RESULTS and DISCUSSION

The Principal component analysis is an analysis method that identifies the direction of the relationship between variables and transforms the original variables in a dataset into less unrelated variables, making them more easily interpretable (Faloye et al., 2024). In this study, the drought stress tolerance of grafted and vermicomposted eggplant plants was evaluated under drought stress conditions. In results of the first 2 basic components for morphological and physiological characteristics are given in Table 1. Accordingly, the first component accounted for approximately 94.40% of the total variation and 96.49% of it could be explained by the first two components. In other words, 96.49% of the total variance of ten variables concentrated on two variables (the principal component). In addition, principal components with a value greater than 1 according to their eigenvalues were considered important. Figure 1 shows the scree plot of ten principal components. This scree plot consists of decreasing eigenvalues that provide visualization of the principal components.

Principal component loads are given in Table 2, and the loading plot created for the plant morphological and physiological characteristics of the first two principal components is given in Figure 2a. The loading plot is interpreted according to the correlation between each variable and each principal component. Accordingly, there is a positive correlation between the close (narrow-angle) variables. There is a negative correlation between the variables that are 180^0 discrete. In addition, there is no correlation between the variables with an angle of 90^0 between them, that is, the variables are independent. According to this, although it is seen that all variables have close effects on each other, it is understood that especially the SDW, SFW, and RWC variables affect the first basic component more. However, the second principal component is further defined by the LA, SD, RDW, and SPAD. There is a negative correlation between PC2 and SDW, LA, RFW, SPAD, and RWC, and a positive correlation between PC2 and the rest (Table 2).

Table 1. Total variance explained for the first two principal components
Çizelge 1. İlk iki temel bileşen için açıklanan toplam varyans

Component	Eigenvalue	% of explained variance	Cumulative variance (%)
1	9.12	91.23	91.23
2	3.19	3.190	94.42

Table 2. Coefficients and loadings of the first two basic components
Çizelge 2. İlk iki temel bileşenin katsayıları ve yükleri

Trait	PC1	PC2
SFW	0.323	0.190
SDW	0.328	-0.084
SL	0.318	0.169
SD	0.313	0.455
LA	0.314	-0.467
RFW	0.316	-0.061
RDW	0.308	0.423
SPAD	0.313	-0.443
g_s	0.306	0.152
RWC	0.323	-0.316

SFW: shoot wet weight, SDW: shoot dry weight, SL: shoot length, SD: shoot diameter, LA: leaf area, RFW: root fresh weight, RDW: root dry weight, SPAD: Chlorophyll SPAD value, g_s: stomatal conductivity, RWC: Relative water content

According to the Bi-Plot, in grafted plants, all treatments of V in the MS condition appear to be correlated with all variables studied. The combination of 'V (3%) + MS' showed a strong and positive correlation with the SPAD, LA, and RWC values. The proximity of this combination especially to SPAD and LA; indicates that 3% V application in MS medium of grafted plants supports strong increases in plant chlorophyll content and LA. Indeed, the results obtained indicate that the leaf's chlorophyll content decreases due to the damage in chloroplasts with the increase in the severity of drought stress, and grafting and V application reduce the losses in leaf chlorophyll content (Kazeminasab et al., 2016; Kıran, 2019b). Greater preservation of chlorophyll pigment, RWC, and LA in grafted plants under drought stress conditions has been reported by Jiao et al., (2023). The delay in the destruction of photosynthetic pigments by V application could be explained as the reduction of oxidative stress with the preservation of osmotic potential in the plant due to the improved water efficiency in the plant. However, the combination of 'V(2%) + MS'; and RWC had positive and very strong correlations with SDW and RFW. This indicates that the application of 2% V in MS medium has a positive effect on the plant growth of the plant. Thanks to the strong root structures of the grafted plants, the leaf water content is preserved by taking more water and nutrients from the stress condition, resulting in SDW and RFW increases that are encouraged. As a matter of fact, the Bi-plot indicates RFW increases of grafted plants in MS medium. The properties of the root that could play an active role in water and ion uptake are directly proportional to the root genotypic properties and total root surface area (Zhang et al., 2020).

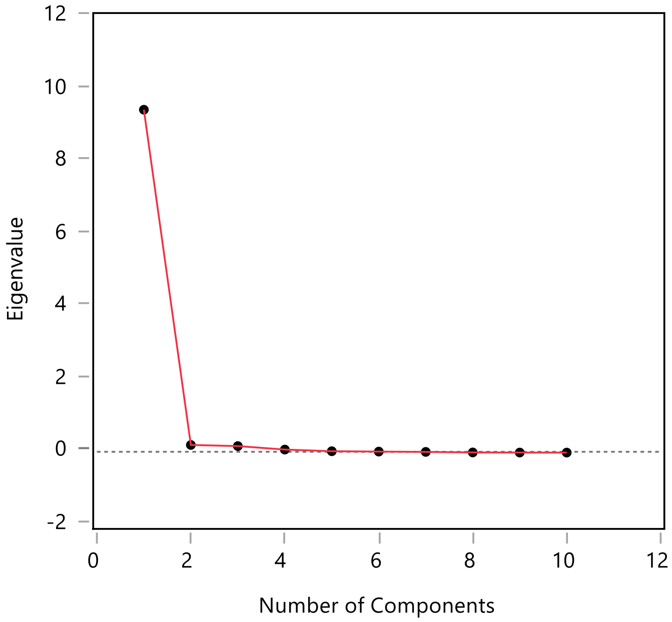

Figure 1. Scree plot of the PCA model
Şekil 1. PCA modelinin eğim grafiği

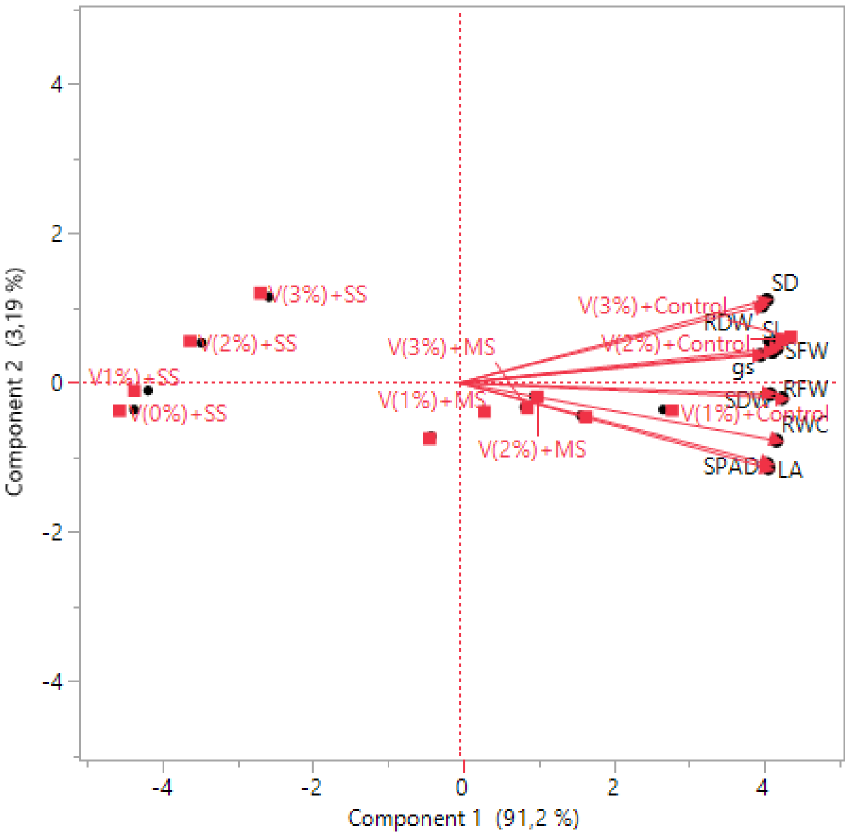

Figure 2. Morphological and physiological features in the Bi-plot explained by the two principal components in grafted plants. SFW: shoot wet weight, SDW: shoot dry weight, SL: shoot length, SD: shoot diameter, LA: leaf area, RFW: root fresh weight, RDW: root dry weight, SPAD: Chlorophyll SPAD value, g_s: stomatal conductivity, RWC: Relative

Şekil 2. Aşılı bitkilerdeki iki ana bileşenle açıklanan Bi-grafikteki morfolojik ve fizyolojik özellikler. SFW: sürgün yaş ağırlığı, SDW: sürgün kuru ağırlığı, SL: sürgün uzunluğu, SD: sürgün çapı, LA: yaprak alanı, RFW: kök taze ağırlığı, RDW: kök kuru ağırlığı, SPAD: Klorofil SPAD değeri, g_s: stoma iletkenliği, RWC: Bağıl su içeriği

It was also noted that V supports plant growth due to its positive effect such as containing growth hormones such as auxin and cytokinin, which increase plant growth, and provide nitrogen fixation (Wong et al., 2020). Similar results were reported by Ghaffari et al. (2022). The acute angles between all variables examined show that the variables are in strong and positive relationships with each other. In particular, the relationships between SPAD and LA, SDW and RFW, g_s and SFW and SL, RDW and SD were found to be quite strong and positive. Growth and development parameters are morphological phenomena that occur with cell growth, elongation, and differentiation (Osakabe et al., 2014). It is a fact that these events occurring at the whole cellular level are related to the loss of turgor that occurs due to the decrease in the relative water content in drought conditions. However, it is stated that the reduction in leaf area leads to a decrease in gas exchange due to a decrease in stomatal opening (Sousa et al., 2022). It is known that grafted plants develop different mechanisms such as accumulating solutes compatible with drought stress tolerance (Munns & Tester, 2008), transmitting some hormones from rootstock roots to the pen (El-Mog et al., 2022), and especially that high root volume is effective in water and nutrient uptake. It is stated that V helps to provide environmental conditions that affect tolerance to stress by increasing water use efficiency in arid conditions, causing changes in soil structure and texture, soil reaction, macro and micronutrient content, and organic matter content (Demir, 2019, 2020; Demir & Kıran, 2020).

CONCLUSIONS

In this study, the drought tolerance of grafted and vermicomposted eggplant plants was evaluated depending on some morphological and physiological parameters, and the positive effect of vermicompost and grafting was confirmed in the current study (Kıran et al., 2023). It is seen that each variable examined in drought tolerance has a different importance. However, PCA, by evaluating morphological and physiological characteristics as independent groups, showed that the variance observed in drought tolerance of grafted and vermicomposted plants could be explained by all variables examined. In addition, the close and positive relationships between morphological and physiological characteristics observed according to PCA confirmed that morphological changes under drought stress are directly related to physiological processes that affect plant growth. As a result, it has been shown that principal component analysis can be used as an effective method to evaluate the effects of grafted plant use and V applications on the drought stress tolerance of eggplant.

Author's Contribution

Author contributions SK designed the study, methodology, and project management, SK, ZD, HFB, KA, SZ, and ŞK performed the experiment and collected and analyzed data. SK wrote and edited the first draft, and ZD, HFB, KA, ŞK, and ŞŞE supported and corrected it. All authors read and confirmed the draft text of the manuscript.

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Statement of Conflict of Interest

The authors of the article declare that they do not have any conflict of interest.

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