

Effects of application of biostimulants and growth regulators on root traits and analysis of root architecture with machine learning in *Lilium*

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Abstract: Apart from the roots, the bulb is the most important organ for plant development of the lily plant. In this experiment, the effects of mycorrhizal, vermicompost and promalin applications on the root architecture of *Lilium* oriental hybrid 'Adelante', a bulbous plant, were to be investigated. It was found that the effect of the treatments on root length (128.6 cm), root surface area (8 cm³), number of tips (111.5), number of forks (354.4) and number of crossings (86.2) was lower than that of the control. In terms of root volume, the applications of vermicompost (3 cm²) and promalin (3 cm³) were the most effective. The most effective application on root diameter (3.5 mm) was promalin. In conclusion, the effect on mycorrhizal root development was lower than the control but higher than other applications. In addition, machine learning (ML) algorithms, including linear regression (LR), sequential minimal optimisation for regression (SMOreg), Gaussian process (GP) and artificial neural network-based multilayer perceptron (ANN-based MLP), were used in the study. The input variables were evaluated for modelling and predicting root traits. The performance values of the ML algorithms were noted in the following order: LR > SMOreg > GP > MLP. These results have important implications for the prediction of root growth in lily crops.

Keywords: *Lilium*; machine learning; mycorrhizal; promalin; root architecture; vermicompost

The lily (*Lilium* spp.) is one of the most widely cultivated flowers in the world. Lily cultivars are widely cultivated for use as cut flowers in ornamental horticulture, often due to their attractive shape, wide range of colours, large number of flowers, and pleasant fragrance, and are highly sought after and valuable worldwide (Liao et al. 2012; Woolf et al. 2012). Experiments on plant nutrition in bulb flowers are generally difficult to conduct. This is because the bulb itself densely stores the nutrients the plant needs, and the soil in which it grows is generally rich in the minerals necessary for accumulating these nutrients (Taiz, Zeiger 2010; Marschner 2012). However, this is usually insufficient during the plant's development. Several studies have shown that a nutrient-deficient environment limits plant

development and nutrient accumulation (Ning et al. 2013; Xu et al. 2017). For this reason, plants should be fertilised to achieve better quality produce (Verma et al. 2021). Additionally, the nutrient requirements, sources, and periods of different plants vary depending on their physiological and biochemical characteristics (Wang et al. 2006; Li et al. 2019). The roots are the most important plant organs for plant nutrition, and under stress conditions, the underground parts of plants are most affected (Zonta et al. 2006; Comas et al. 2013; Bucksch et al. 2014). A strong root structure increases crop yields by enhancing water and nutrient uptake, as well as improving resistance to diseases and pests. Root length and root volume are key indicators of a plant's ability to absorb water and nutrients (Zonta et al. 2006;

Bayındır, Kandemir 2023). Mycorrhizae are sources of nitrogen, phosphorus, potassium, and other minerals for plants, and the presence of these minerals promotes growth (Garcia, Zimmerman 2014; Hijri, Bâ 2018; Yeh et al. 2019). Various studies have found that mycorrhizae improve the root and stem structure of ornamental plants (Bhattacharyya, Jha 2012; Tariq et al. 2016; Püschel et al. 2017; Jochum et al. 2019). Vermicompost is a humic substance with effects similar to those of growth regulators. The positive hormonal effect of vermicompost has been established thanks to the ability of worms to secrete compounds such as auxin, cytokinin and gibberellin, which play a role in plant development (Yilmaz 2017; Baruati et al. 2018). Studies have been conducted on the joint use of vermicompost and mycorrhiza, and it has been found to increase plant yield and nutrient uptake. As a result, its use in crop production was considered beneficial (Küçükymuk et al. 2014). Vermicompost applied to *Lilium* Asiatic hybrid var. 'Navona' plants have been shown to improve growth and development by increasing the number and length of roots (Moghadam et al. 2014). There are few studies on bulb and root development of gibberellic acid (GA₃) and cytokinin; however, the effects of promalin applications on bulb growth, especially on root architecture properties, are under-researched.

Since the root structure is underground by nature, it is not very easy to study. For this reason, there are only a few studies that use the phenotypic traits of the root as a basis. In recent years, significant progress has been made in measuring roots. There are now methods for analysing plant images that are simpler, faster, repeatable and more descriptive of root growth (Judd et al. 2015; Paez-Garcia et al. 2015). In addition, modelling techniques for root trait structure and activity based on multivariate and ML techniques have been investigated. However, further studies are needed to determine the importance of root traits in influencing aboveground biomass (Moon et al. 2018; Awika et al. 2021; Tütüncü 2024). The use of ML in plant science is of growing interest. ML is used to predict the impact of various applications in agriculture, particularly on crop yield. ML algorithms such as support vector machines (SVM), random forest (RF), and multilayer perceptron (MLP) are used to analyse large datasets in plant breeding programs to improve efficiency and develop model-based breeding methods (Yoosefzadeh-Najafabadi et al. 2021). WEKA, a ML

workbench, offers a collection of state-of-the-art ML algorithms and data preprocessing tools suitable for data mining applications (Frank et al. 2010).

In lilies, the bulbs together with the roots are the most important organs for the development of the plant. However, the effects of growth-promoting applications, such as mycorrhiza, vermicompost and promalin on the root architecture of lilies are not well understood. Therefore, the aim of this study was to evaluate the effects of two different biostimulants, mycorrhiza and vermicompost, and promalin, a plant growth regulator, on root architecture characteristics in the soilless culture of *Lilium* oriental hybrid 'Adelante'. The effects of the applications on the architectural features of the roots were evaluated using image analysis. In addition, the study attempted to model and predict the impact of the applications on root architecture by using methods such as artificial neural network analysis and ML based on data mining. This study also aims to provide growers with best practices for root development.

MATERIAL AND METHODS

The study was carried out between April 15 and October 30, 2023 at Samsun Black Sea Agricultural Research Institute, Samsun, Türkiye (41°13'51.63"N, 39°29'47.30"E).

Plant material. The *Lilium* oriental hybrid 'Adelante' was used as plant material in the study. It produces pink flowers and is a fragrant variety. The bulbs purchased from a commercial company were planted on April 15, 2023, in 5-litre pots with a culture medium of peat and perlite (3 : 1). Before planting, the bulbs were sprayed with a fungicide (Figure 1). The study used 20 bulbs for each treatment, with 20 repetitions for each, and each repetition contained a single plant in a pot.

Climatic conditions. The average temperature in the greenhouse was 25.6 °C, and the humidity in 2023 was 68%. During the experimental period, the total sunshine duration from January 1 to December 31, 2023, was 1 150 hours.

Experimental setup and treatments. The applications were made on May 1, 2023, when the plants entered their active growth phase (reaching a height of 10 cm), and the plants were sprayed once. No application was made to the control group. At the application stage, the mycorrhiza (M) was weighed at 5 g and mixed with 1 L of water. Vermicompost (V) was



Figure 1. Blooming *Lilium* oriental hybrid 'Adelante'

prepared at a rate of 5 mL per 1 L of water. Promalin (P) was prepared at 500 ppm per litre. M and V were poured into each pot as 50 mL of liquid on the surface of the pot. P was sprayed using a hand pump so that all parts of the plants were wetted. One subject that was not sprayed was included in the experiment as a control. The M, V and P used in the experiment were purchased from commercial companies. The analysis results of the fertilisers declared by the commercial companies are listed in Table 1.

Measurements of root architecture. The root architecture of the *Lilium* oriental hybrid 'Adelante' was analysed using the root analysis tool WinRhizo root analysis software version 2013 (Regent Instruments, Québec City, Quebec, Canada). The lily root system, compared to other flower bulbs, consists of a basal root system and a stem root system. Basal

roots grow in the basal plate at the bottom of the bulb, while stem roots grow underground as fibrous roots between the base of the shoot and the top of the bulb. Basal roots growing at the base of the bulb were used in this study. After the upper parts of the plants had faded entirely, they were removed from the pots on September 30, 2023. The roots of the removed plants were carefully washed and cleaned. The roots were then transferred to the computer in three dimensions using the device's scanner (Epson Expression 10000XL, Epson America Inc., Long Beach, CA, USA). The following parameters of the root structure and the degree of rooting were examined using the WinRhizo software: total root length (cm), root surface area (cm²), root volume (cm³), average root diameter (mm), number of tips, number of forks and number of crossings were calculated using WinRhizo software.

Modelling procedure. To model and predict the root characteristics of *Lilium* after the application of M, V and P, the results obtained by applying different data mining algorithms in the WEKA 3.9.6 software (Machine Learning Group, College of Waikato) (Bouckaert et al. 2016) to the dataset were compared. A model was created by selecting the algorithm with the highest success rate among these algorithms. Four machine learning (ML) methods were used in the study: multilayer perceptron (MLP), linear regression (LR), sequential minimal optimisation for regression (SMOreg) and Gaussian process (GP). To fully evaluate the performance of the models, we used 10-fold cross-validation to split the dataset into training and test subsets (70 : 30). The input variables consisted of one species and seven different root characteristics were measured (root length, root surface area, root volume, average root diameter, number of tips, number of forks and number of crossings). The target variables (output) control, M, V, P, and P + M were included (Figure 2).

Table 1. Fertiliser ingredients declared by commercial companies

Application	Trade name	Ingredients
Mycorrhizal preparat	Endo Roots Soluble (ERS)	total living organism 1 × 104 w/w, contained organisms: <i>Glomus intradices</i> , <i>Glomus aggregatum</i> , <i>Glomus mosseae</i> , <i>Glomus clarum</i> , <i>Glomus monosporum</i> , <i>Glomus deserticola</i> , <i>Glomus brasiliense</i> , <i>Glomus etunicatum</i> , <i>Gigaspora margarita</i>
Promalin	Sumitomo chemical/Promalin	18.5 g/L gibberellin GA ₄₊₇ + 18.8 g/L 6-benzyladenine
Vermicompost	ORPEX	total organic matter 6%, total nitrogen 0.5%, organic nitrogen 0.2%, water-soluble potassium oxide (K ₂ O) 0.2%, phosphorus pentoxide (P ₂ O ₅) 0.05%

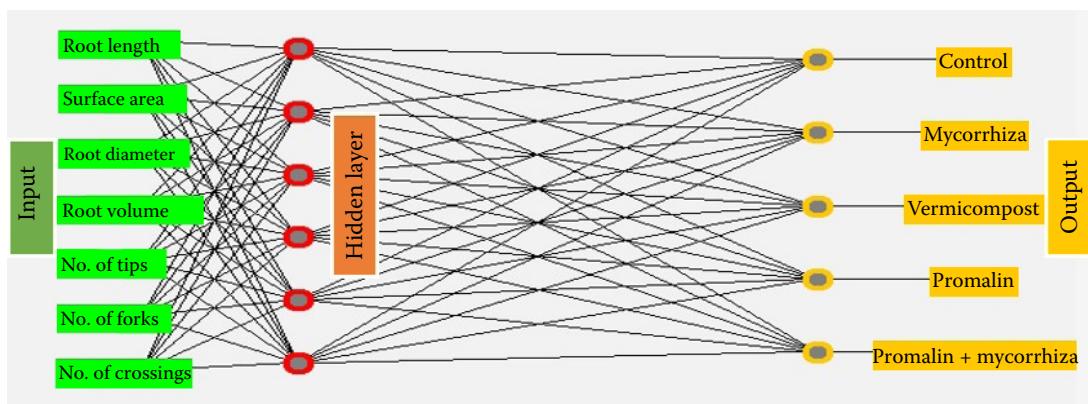


Figure 2. The multilayer perceptron (MLP) structure with seven inputs, five outputs, and six hidden neurons

Data evaluation. The study was conducted according to a completely randomised design with 20 replicates. Each replicate consisted of a single plant. Analysis of variance (ANOVA) was performed using SPSS statistical software version 20.0, and differences between treatments were compared using Duncan's multiple range test (within 5% and 1% significance levels).

RESULTS

Properties of the root architecture. The effects of the applications on the architectural properties of the roots were found to be statistically significant. The treatments with M, V, P and P + M reduced root length by 31%, 49%, 55% and 44%, root surface area by 25%, 36%, 38% and 43%, number of tips by 47%, 60%, 66% and 52%, number of forks by 46%, 53%, 63% and 56% and number of crossings by 70%, 73%, 90% and 74%, respectively, which was lower than the control (Table 2, Figure 3). The root vol-

ume was increased by the application of V, P, and P + M by 50%, 50%, and 25%, respectively, while the M application showed the same effect as the control. While the root diameter was 33% lower in M and V applications compared to the control, it was 50% lower in P + M applications. P, on the other hand, increased root diameter by 17%. Root length, root surface area, number of tips, number of forks, and number of crossings were lower in all treatments compared to the control. Among the treatments, P showed the lowest values for root length, number of tips, number of forks, and number of crossings. In contrast, both P and V increased root volume to 3.0 cm^3 , representing a 50% increase compared to the control. P was also the only treatment that increased root diameter (3.5 mm), whereas the lowest diameter value (1.5 mm), corresponding to a 50% reduction, was observed in the P + M treatment (Table 2, Figure 3).

Machine learning (ML) modelling analysis. The research results were analysed using ANN-based MLP, LR, SMOreg and GP. The results were vali-

Table 2. Effect of the applications on the properties of the root architecture

Applications	Root length (cm)	Root surface area (cm^2)	Root volume (cm^3)	Root diameter (mm)	Number of tips	Number of forks	Number of crossings
Control	128.6 ^a	8.0 ^a	2.0 ^b	3.0 ^{ab}	111.5 ^a	354.4 ^a	86.2 ^a
M	88.2 ^b	6.0 ^b	2.0 ^b	2.0 ^b	57.9 ^b	192.8 ^b	25.7 ^b
V	66.0 ^c	5.1 ^{bc}	3.0 ^a	2.0 ^b	43.7 ^c	167.4 ^c	23.1 ^b
P	57.7 ^d	5.0 ^{bc}	3.0 ^a	3.5 ^a	37.1 ^d	129.8 ^d	8.5 ^c
P + M	71.6 ^{bc}	4.6 ^c	2.5 ^{ab}	1.5 ^c	52.4 ^b	155.2 ^c	22.2 ^b
	**	*	*	*	**	**	**

M – mycorrhizal; V – vermicompost; P – promalin; P + M – promalin + mycorrhizal

*, **significance at $P \leq 0.05, 0.01$, respectively

^{a–d}means with different letters within the same column indicate significant differences (Duncan's multiple range test)

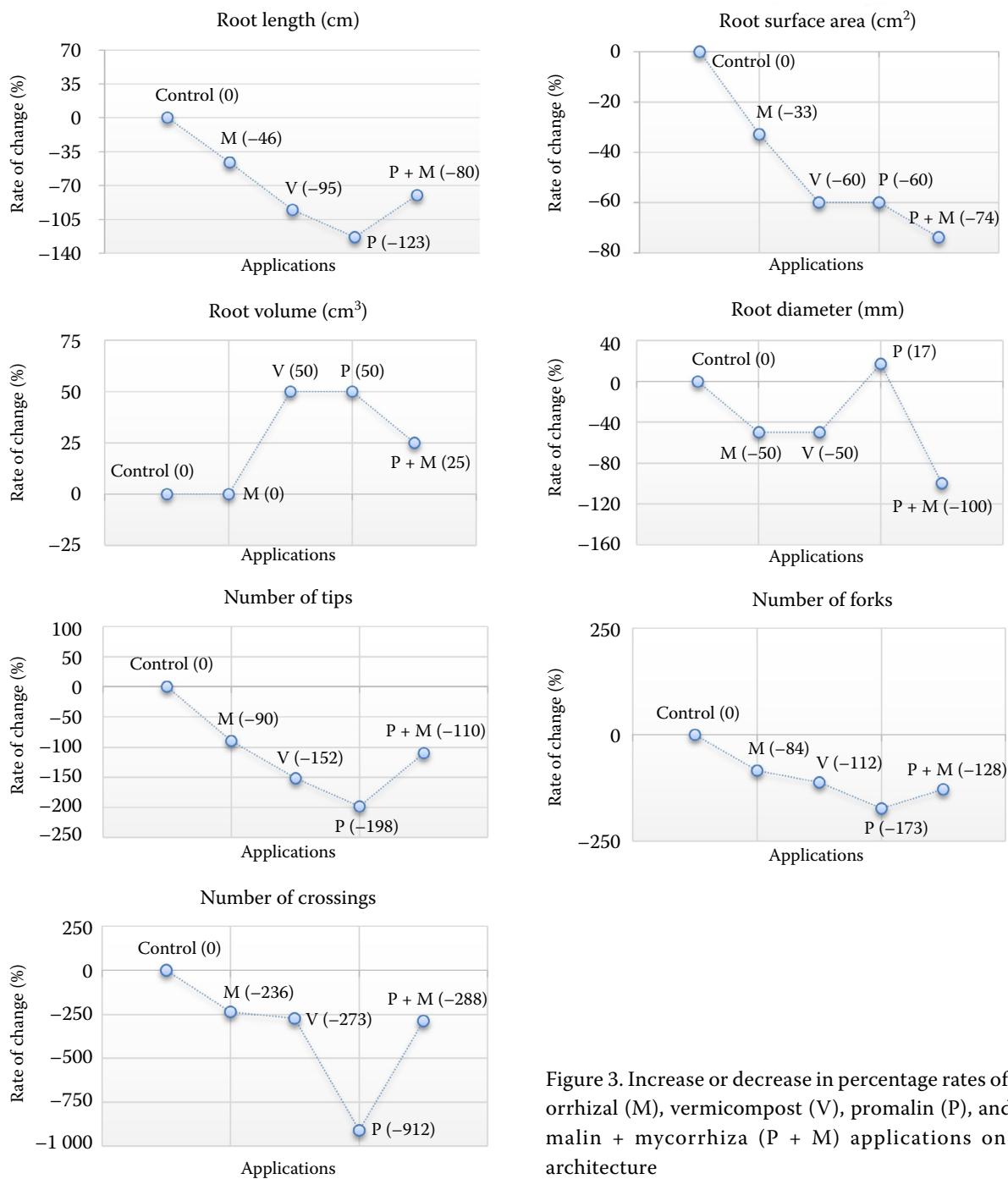


Figure 3. Increase or decrease in percentage rates of mycorrhizal (M), vermicompost (V), promalin (P), and promalin + mycorrhiza (P + M) applications on root architecture

dated and estimated using three performance metrics: root mean square error (RMSE), R -squared (R^2) and mean absolute error (MAE). The LR model showed varying R^2 values between 0.88 and 0.99. The highest R^2 value was observed for root length and number of tips at 0.99, while the lowest value was recorded for number of crossings at 0.88. MAE values are generally low, ranging from 0.03 to 0.12. While the number of crossings exhibited the highest RMSE of 0.21, the root length and number of tips

had the lowest RMSE values (Figure 4). The R^2 values for the SMOreg model varied between 0.89 and 0.99, with the highest R^2 value of 0.99 for root length, number of tips, and number of forks, and the lowest value of 0.89 for surface area. The MAE values of the SMOreg model are generally low, ranging from 0.03 to 0.16. While the highest RMSE value of 0.16 was attributed to the surface, root length, number of tips and number of forks had the lowest RMSE value of 0.02 (Figure 5). The R^2 values for the

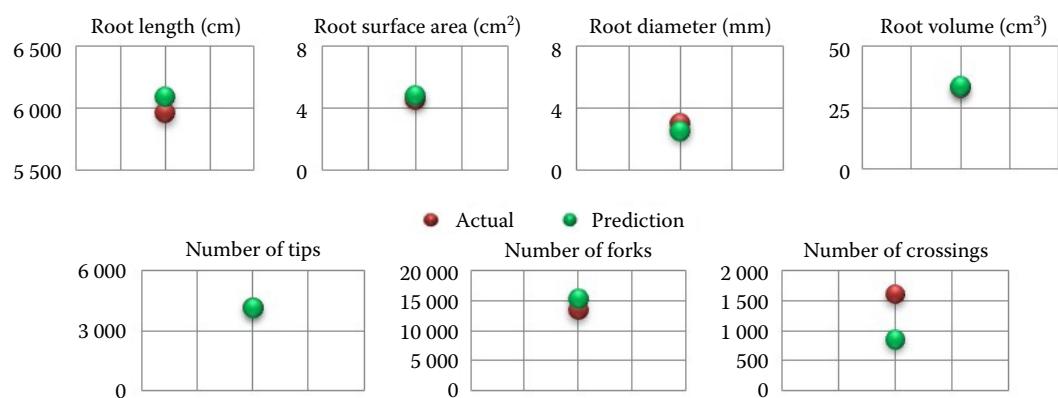


Figure 4. Observed and predicted values of the parameters using the linear regression (LR) model

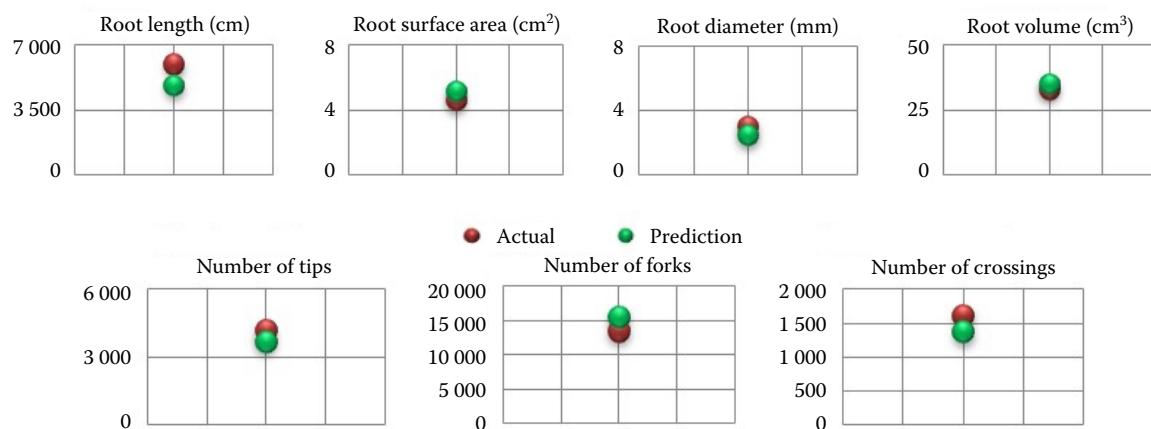


Figure 5. Observed and predicted values of the parameters using the sequential minimal optimisation for regression (SMOreg) model

GP model were very close to those of the MLP model, ranging from 0.66 to 0.99. The highest R^2 value was measured for the number of tips and root length at 0.99 and 0.98, respectively, while the lowest value was measured for surface area at 0.66. The MAE values were generally low; the number of tips had the

lowest value at 0.02. The RMSE values ranged from 0.02 to 0.18, with surface area having the highest RMSE value and the number of peaks the lowest (Figure 6). The R^2 values for the MLP model ranged from 0.41 to 0.99 in comparative assessments of different root system characteristics using these models and

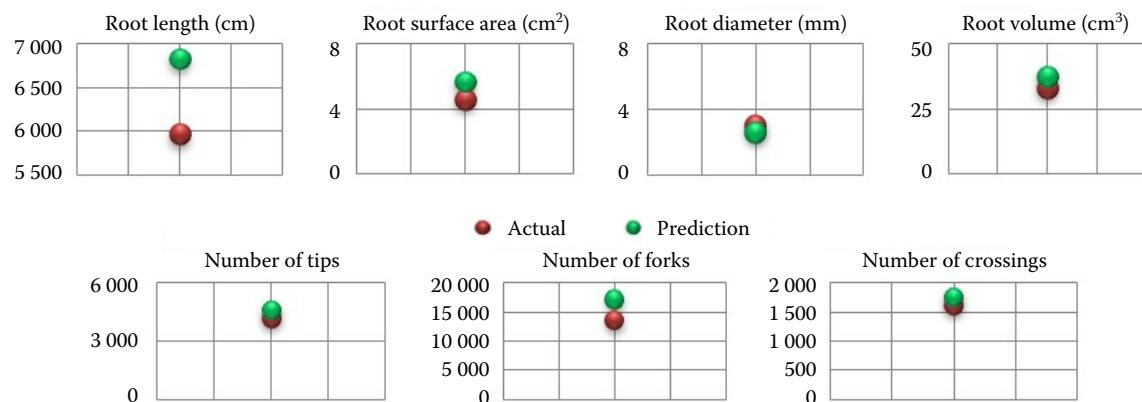


Figure 6. Observed and predicted values of the parameters using the Gaussian process (GP) model

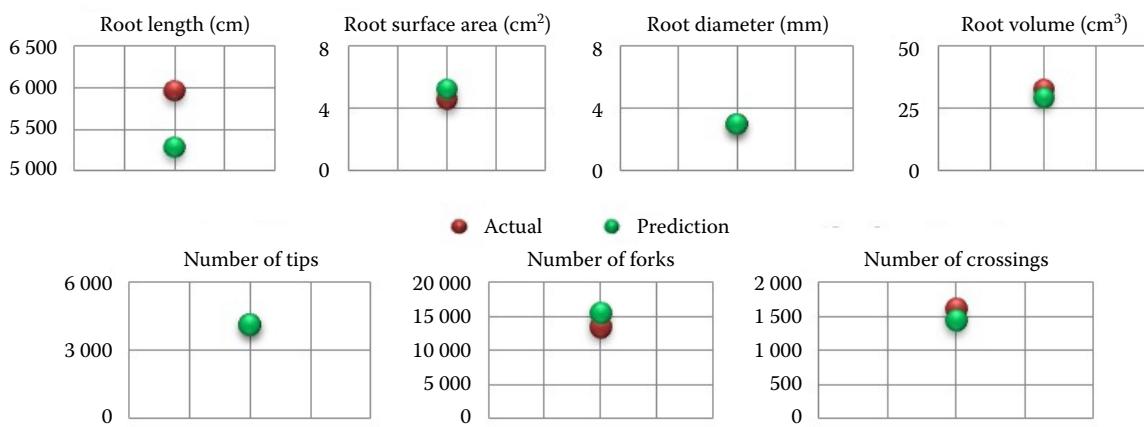


Figure 7. Observed and predicted values of the parameters using the multilayer perceptron (MLP) model

their performance measures. The highest R^2 value was found for root length and number of crossings at 0.99 and 0.98, respectively, while the lowest value was found for volume at 0.41. The MAE values were generally low, with root length and number of crossings having the lowest values at 0.04. The RMSE values ranged from 0.03 to 0.30, with volume having the highest RMSE value, while root length and number of crossings had the lowest (Figure 7). The R^2 ratios of the WEKA algorithms were found to be in the following order: LR > SMoReg > GP > MLP.

DISCUSSION

Impact on the properties of the root architecture.

The influence of all applications on the properties of the root architecture was found to be statistically significant in this study. Root length, root surface area, number of tips, number of forks and number of crossings were lower with the application of M, V, P and P + M than with the control. While root volume increased with V, P and P + M applications compared to the control, the effect of M application was similar to the control. While the root diameter was lower in the M, V and P + M applications than in the control, it was higher in the P application than in the control. The application with the lowest root diameter was the P + M application. While root length, root surface area, number of tips, number of forks and number of crossings were lower in all treatments than in the control, it was found that the application with the lowest values was the P application. On the other hand, root volume was higher in both P and V and P + M applications

than in the control. According to Alkaç et al. (2022), in their study on *Dahlia variabilis*, the application of M reduced root length by 24% compared to the control. In addition, the application of 100 mL/L of P was reported to strongly inhibit root initiation and growth in Asiatic lily cultivars 'Golden', 'Coral' and 'Peach' (Zhang et al. 1989). In general, one of the reasons for the poor results in this study is the assumption that the treatments have a physiological effect on the plant body. In fact, M treatments are thought to cause changes in plant morphology and physiological structure as well as in the chemical composition of plant tissue (Smith, Read 2010). In terms of the properties of the applications, there have been many studies in the past showing that M, V and P applications support plant nutrient uptake (Bachman, Metzger 2008; Garcia, Zimmermann 2014; Moradi et al. 2014; Roushaphel et al. 2015; Trouvelot et al. 2015; Balode 2017; Hijri, Bâ 2018; Yeh et al. 2019). Similarly, the hormone-like activities of V have been reported to increase rooting, root biomass and root number (Alvarez, Grigera 2005; Bachman, Metzger 2008). In this study, it was concluded that the applications facilitated the plants' access to nutrients and water; however, they did not enhance the plants' root development to reach these resources. The control plants showed normal growth as they had no support. Although the root volume was the same in the M application as in the control, it increased when V, P and P + M were applied. In contrast to root length, this increase is not an expression of efforts to reach the plant nutrients. On the contrary, it indicates that plants increase their root volume to enhance nutrient uptake. It has even been suggested that root length and root vol-

ume are important indicators of a plant's ability to take up water and nutrients (Zonta et al. 2006). It has also been shown that the longer the roots, the better the access to nutrients and water (Zonta et al. 2006). Similarly, inoculation with M has been reported to improve the rooting capacity of plants (Karaçal, Tüfenkçi 2010).

It is known that the root diameter influences the absorption capacity of the plant. Plants with smaller root diameters have a higher absorption capacity (Sarı, Çelikel 2021). However, the application of P alone increased both root diameter and root volume, but it was found that this treatment resulted in the least development of other root traits. These results indicate that the application of P has a negative effect on the root development of *Lilium*, a bulbous plant. In fact, the effects of gibberellin and cytokinin in the composition of P mainly promote the elongation, branching and flowering of the plant. Gibberellins increase internode elongation and promote flowering in many plants (Khan, Chaudhry 2006). Additionally, GA₃ applications were found to increase the amount of nitrogen in the leaves, promoting vegetative development (Yeşiloglu 1988). Cytokinin was found to play a regulatory role in the activity of the plant meristem in the shoot (Werner et al. 2001). Based on these results, it was concluded that the bulb, which is a food source in P-treated lilies, rearranges the assimilate distribution and directs more assimilate to the upper part of the plant and less assimilate to the root development. In addition, P has been reported to have an anti-ageing effect on plants by reducing the effect of ethylene (Çelikel et al. 2002). This result supports the assumption that the plant requires more energy to protect its green parts, and therefore, more assimilation is shifted to the upper part. In this study, the reduction in root diameter of *Lilium* to increase nutrient uptake is consistent with studies reporting that root diameter is reduced in the vegetative development phase when there is nutrient deficiency or excess in the medium. In fact, a high percentage of fine roots has been reported to be a desirable trait for plants (King et al. 2002; Stokes et al. 2009). However, the lower reduction in root diameter in P applications compared to other applications indicates that the plant also requires energy to reduce root diameter. The ability of plants to take up nutrients has been found to increase significantly with increasing numbers of roots, tips and crossing densities (Craine 2006). However, this study found that these increases de-

pend on the applications and the physiological and morphological effects of these applications on the plant. It was concluded that the applications in this study reduced root growth, depending on the assimilated distribution of the plant, with the effects being more related to bulb development. The control plants exhibited balanced development, as no intervention was made.

Root system development is intrinsically linked to flowering success. An intact root system enhances nutrient uptake and water absorption, both of which are essential for supporting flower development. Increased root volume resulting from treatments such as V or P is positively correlated with improved flower quality. As studies have shown, larger root systems can enhance overall plant health and vitality, leading to more abundant and higher-quality flowers (López-Bucio et al. 2003; Giehl et al. 2014; Sun et al. 2017; Al-Ajlouni et al. 2023). Future research should continue to explore these relationships to develop effective strategies for elucidating the relationships between flowering and root architecture in *Lilium* species.

Performance of modelling the architecture of the root system. In this study, MLP, LR, SMoReg and GP were used to estimate the effects of M, V and P applications on root architecture. All models with R^2 values between 0.89 and 0.99 were based on root length, number of tips, and number of forks and showed high performance for parameters such as number of crossings. While the models performed well for most of the evaluated parameters, root length showed the best performance across all models. The models performed worst for root volume and root surface area. It is possible that the input variables cannot fully explain the behaviour of the mentioned parameter (Duarte et al. 2022). WEKA is frequently used for classification tasks in various fields, including plant-related studies. The accuracy of WEKA in classifying different plant species varies depending on the algorithms used. For example, the J48 algorithm in WEKA achieved an overall accuracy of 99.9973% in a study on power quality issues (Kiranmai, Laxmi 2018). In Tütüncü's (2024) study on *Primula*, the MLP and GP models demonstrated the best performance among the models when considering all parameters. In contrast, XGBoost showed the worst performance among the other models, with low R^2 values.

When predicting soybean yields from hyperspectral reflectance data, the random forest (RF) algorithm demonstrated the highest individual per-

formance, achieving an 84% yield classification accuracy. This allows farmers to optimise their inputs and predict production (Yoosefzadeh-Najafabadi et al. 2021). When the WEKA tool is used for classification algorithms, accuracy rates vary for ornamental crops. In the study of the soil monitoring and recommendation system for ornamentals, a high accuracy rate of 89.6% was achieved using an 80 : 20 data split (Angdresey et al. 2021). In this study, similar to the researchers' results, different accuracy rates were achieved with different algorithm applications. In this study, a 70 : 30 data split was used, and an accuracy of 99.89% was achieved. This may allow manufacturers to optimise input and predict production. In conclusion, ML in agriculture offers a data-driven approach to optimise crop yields by enabling yield prediction, precision farming methods, efficient resource management, and the integration of crop modelling with ML for improved predictions and decision-making (Shahhosseini et al. 2021).

CONCLUSION

The architectural features of roots and their responses to various conditions enable plants to adapt to different conditions. In order to obtain quality products in ornamental horticulture, it is very useful to know the effects of different applications on root development. All treatments had a negative effect on root length, root surface area, the number of tips, the number of forks, and the number of crossings. In this study, it was concluded that the treatments facilitated the plants' access to nutrients and water, and therefore did not promote root development in plants with access to nutrients and water more than the control. The control plants showed normal growth as they had no support. When the treatments were evaluated individually, the M application showed a less pronounced reduction in root architectural traits compared with the other treatments. However, compared with the control, M still caused a moderate decrease in most root characteristics. In addition, the treatments may have influenced assimilate allocation, contributing to reduced root growth, so that root development tended to decrease. The results of this study also show that the nutrient requirements of bulb and non-bulb plants are different in relation to the goals of plant development. Therefore, it would be beneficial to conduct more species- and cultivar-specific studies.

Overall, the ML methods showed sufficient potential to predict the root architecture of *Lilium* oriental hybrid 'Adelante' cultivar, especially LR and SMOreg, with better values for R^2 , MAE and RMSE. Moreover, these algorithms allowed us to evaluate the input variables. Among the input variables, root length, number of tips, number of forks and number of crossings were the most important parameters when using ML algorithms. The result of this work was the simplification of the prediction process, which required only a single model to predict the root architecture features under investigation. It can be concluded that ML models, especially LR and SMOreg, can effectively predict the root architecture variables of *Lilium* oriental hybrid 'Adelante' cultivar.

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