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Evaluation of five rootstocks on the growth, gas exchanges and chlorophyll fluorescence of *Juglans regia* L. cv. 'Xiangling'

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Abstract: Our study comparative analyzed the morphological and physiological performance of *Juglans regia* L. cv. 'Xiangling' (*JRX*) grafted onto five rootstock species originated from China. A wide range of coefficient of variation (CV), 1.155–39.848%, was detected for fourteen indexes among the studied seedlings. CV of plant height, total dry biomass, total leaf area, root/shoot ratio, chlorophyll index, and stomatal conductance was higher than 15.00%, suggesting higher variation. Principal component analysis, hierarchical cluster analysis and subordinate function analysis were used to screen engrafted seedlings for their quality. All three methods produced the same result that seedling quality of 'Xiangling' engrafted with the five rootstock species from high to low were – *Juglans regia* L. cv. 'Jizhaomian' (*JRJ*), *JRX*, *Juglans regia* L. cv. 'Lvxiang' (*JRL*), *Juglans mandshurica*, Maxim., and *Juglans hopeiensis* Hu.. The evaluation results were highly consistent with the actual growth performance of the engrafted seedlings, it verified the reliability of our results and the evaluation methods. The results demonstrated that *JRJ*, *JRX*, and *JRL* are potential rootstock candidates in the studied walnut planting regions. It would provide important implication for the selection of proper rootstock species for a certain particular walnut variety.

Keyword: gas exchange; growth performance; multivariate analysis; walnut rootstock

Persian walnut (*Juglans regia* L.), one of the oldest cultivated edible nut species originating from Central Asia, is widely cultivated in the temperate regions of the world including Eastern Asia, Northern Africa, USA and Western South America (Bernard et al. 2018). According to the FAO (2021), China is the largest producer of walnut in the world. As one of the most important national strategic tree species, walnut is widely planted in the rural areas of China and playing an increasingly significant role in adjusting the structure of agricultural planting, increasing farmers' income, guaranteeing national grain and oil security and ecological security, and earning foreign exchange from export. However, walnut industry in China has entered a bottleneck period mainly due to the large plantation in shallow hilly and mountainous areas with poor soil, long-term use of seedling-plants or traditional rootstocks that resulted in low yield and poor quality (Liu et al. 2020). Therefore, selection of suitable walnut rootstock and multiplication of commercial variety engrafted seedlings with high quality are the critical step in walnut

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industry, which are the primary premise for high survival rate of planting, well vegetative growth performance, early fruiting, high yielding and quality, and ultimately high economic benefit (Gauthier, Jacobs 2011; Albacete et al. 2015; Rasool et al. 2020; Sharma et al. 2020; Vahdati et al. 2021).

Grafting is a widely used effective way to realize variety cultivation and vegetative propagation in agriculture, horticulture and silviculture (Schwarz et al. 2010; Baron et al. 2019; Rasool et al. 2020). Rootstock has an important effect on the scion vegetative growth by influencing the absorption/synthesis and transportation of water, nutrients and hormones (Nawaz et al. 2016; Warschefsky et al. 2016; Gaion, Carvalho 2017; Rasool et al. 2020; Thapa et al. 2021; Vahdati et al. 2021; Mostakhdemi et al. 2022). In addition, rootstocks with high stress tolerance are widely used to improve resistance of the engrafted plants to biotic and abiotic stresses, that was mainly attribute to the well-developed root system, the enhanced availability of water and nutrients, the production of phytohormones, the activated antioxidative defense system, and the up-regulated expression of genes related to stress tolerance (Schwarz et al. 2010; Gaion, Carvalho 2017; Kumar et al. 2017; Rouphael et al. 2018; Gaut et al. 2019; Vahdati et al. 2021; Sadeghi-Majd et al. 2022).

Generally, plant height, basal diameter, dry biomass, leaf photosynthetic capacity and chlorophyll fluorescence characteristics are always been used as important index to evaluate the quality of walnut seedlings or engrafted seedlings (Liu et al. 2020; Shabbir et al. 2021; Arab et al. 2022). In our pervious study, we have comprehensively evaluated the seedlings' quality of six walnut rootstock species originated in China, during which we comparative analyzed the seed germination and the morphological and physiological performance of the seedlings (Liu et al. 2020). Although it provided a significant basis for the selection of rootstocks, researches about the effects of the selected potential rootstocks on growth, fruiting, fruit quality, and stress tolerance of the engrafted trees are urgently needed.

In the present study, we comparative analyzed the morphological and physiological performance of *Juglans regia* L. cv. 'Xiangling' walnut grafted onto five rootstock species originated from China. The main objectives of this investigation were (1) to describe the variability in morphological and physiological levels among 'Xiangling' with different rootstock species; (2) to test the utility of principal component analysis (PCA), hierarchical cluster analysis (HCA) and subordinate function analysis (SFA) in screening engrafted seedlings for their quality; and (3) to identify the potential rootstock species suitable for extensive use in Shandong Province, China.

MATERIAL AND METHODS

Plant material and experimental site. Two-year plants of *Juglans regia* L. cv. 'Xiangling' grafted onto five rootstock species including *Juglans regia* L. cv. 'Jizhaomian' *Juglans regia* L. cv. 'Xiangling', *Juglans regia* L. cv. 'Lvxiang', *Juglans mandshurica* Maxim. and *Juglans hopeiensis* Hu. were studied on their morphological and physiological characteristics. Seeds of the rootstocks were collected from plants growing in their native regions in China (Table 1). The study was conducted at the experimental nursery of Shandong Academy of Forestry, Jinan, Shandong Province, China (36°40'N, 117°00'E). The climate is sub-humid warm temperate continental monsoon climate. Mean annual precipitation and mean annual temperature are 697 mm and 14.1 °C, respectively. The soil in the nursery is cinnamon soil (pH = 7.81), the contents of alkali-hydrolyzable nitrogen, available phosphorus, available potassium, and organic matter in the soil are 50.29 mg/kg, 27.38 mg/kg, 56.17 mg/kg, and 10.55 g/kg, respectively.

Pre-germination, sowing, and seedling management of the five rootstock species followed the methods of Liu et al. (2020). In March of the following year, seedlings of the five rootstock species with uniform growth were selected to graft the scions of 'Xiangling' walnut variety using bud grafting method.

Growth. Before the leaves fall in October, the final plant height (PH) and basal diameter (BD) were recorded from five repeating groups (ten seedlings for each repeat group) of 'Xiangling' grafted on each rootstock species. PH was measured with a tapeline (accuracy – 0.1 cm) from the base to the terminal bud of the main stem; BD was measured with a digital micrometer (accuracy – 0.001 mm) at the base of the main stem. Then, seedlings with different rootstock species were harvested and divided into leaf, stem and root portions. Total leaf area (TLA) was measured with an area meter (AM-100, Analytical Development Company, Hertsfordshire,

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Table 1. Origins in China for the five walnut rootstock species used in the study

Rootstock	Abbreviation	Description
Juglans regia L. cv. 'Jizhaomian'	*JRJ*	It is a unique wild walnut resource in China and often been used as rootstock due to its high resistance to drought, cold and disease. It belongs to sect. *Dioscaryon* and was widely planted in the south mountain regions in Jinan City, Shandong province, China.
Juglans regia L. cv. 'Xiangling'	*JRX*	It is an early-fruiting walnut variety bred by Shandong Institute of Pomology in 1980s. It belongs to sect. *Dioscaryon*. It was widely planted in Northern China and Northwest China and always been used as self-rooted rootstock in China.
Juglans regia L. cv. 'Lvxiang'	*JRL*	It is an early-bearing fresh-edible walnut variety bred by Shandong Academy of Forestry in 2010s. It belongs to sect. *Dioscaryon* and was now widely planted in Northern China.
Juglans mandshurica, Maxim.	*JM*	It belongs to sect. *Cardiocaryon* and is indigenous to China. It is one of the rare tree species in Northern China and Northeast China, and is often been used as vigorous rootstock to enhance drought and cold tolerance, and to improve plant growth.
Juglans hopeiensis Hu.	*JH*	It belongs to sect. *Cardiocaryon* and is indigenous to China. It is narrowly distributed in Northern China in the hilly, mid-elevation area between Hebei province, Beijing, and Tianjin.

UK). After that, the three portions were oven-dried at 60 °C to a constant weight and the dry biomass of each portion were measured, total dry biomass (TDB) was defined as the sum of the three parts. The root/shoot ratio (RSR) was calculated as root dry biomass divided by aboveground (leaf and stem) biomass.

Leaf chlorophyll index (SPAD) and gas exchange. SPAD was determined using a chlorophyll meter (SPAD–520Plus, Konica Minolta, Tokyo, Japan) on the light-directed leaves. On a sunny morning (9.00 a.m. to 11.00 a.m.), gas exchange parameters, including net photosynthetic rate (P_n), transpiration rate (T_r), stomatal conductance (g_s) and intercellular carbon dioxide concentration(C_i), were measured on the light-directed leaflets in the middle of the fully expanded compound leaves using a portable photosynthesis system (LI-COR 6400XT, LI-COR Inc., Lincoln, NE, USA). The light intensity, ambient water vapor pressure, leaf temperature, and CO_2 concentration were set at 1 500 μmol/m/s PPFD (as provided by a blue-red diode Q-Beam light source), 1.30 kPa, 26.00 °C, and 400 μmol/m/s, respectively.

Chlorophyll fluorescence. The chlorophyll fluorescence parameters of newly expanded leaves were determined using a pulse modulated fluorometer (FMS–2, Hansatech, UK) on a sunny day (09.00 a.m. to 11.00 a.m.). After the leaves were adapted to darkness for 30 minutes, the minimum fluorescence (F_0) and maximum chlorophyll fluorescence yield (F_m) were measured and the maximum photochemical efficiency of PSII [$F_v/F_m = (F_m - F_0)/F_m$] were then calculated. Afterwards, the steady state fluorescence yield (F_s'), maximum fluorescence (F'_m), and minimum fluorescence (F'_0) were measured on the same leaves in the light-adapted state. The effective PSII quantum yield ($\Phi PS_{II}) = (F_m' - F_s')/F_s'$ and photochemical quenching (qP) were calculated as and $qP = (F_m' - F_s')/(F_m' - F_0')$, respectively.

Statistical analyses. The experiments were performed through a completely randomized design. All the measurements were conducted in quintuplicate. Data were presented as means of five replicates ± standard deviation (SD). Data processing and statistical analysis were carried out using Microsoft Excel 2013 and SPSS–20.0 for Windows statistical software package (Standard released version 20.0 for Windows; SPSS Inc., Chicago, IL, USA). Analyses of one-way variance (ANOVA) were performed to evaluate the effects of rootstock species on the studied variables. Tukey's HSD (honestly significant difference) post hoc test ($P \leq 0.05$) was performed to test the existence of statistical differences for the same parameter among seedlings with different rootstock species. PCA, HCA, and SFA were performed to comprehensively evaluate the seedling growth performance of 'Xiangling' with different rootstock species.

The subordinate function value (S) for each index of 'Xiangling' engrafted with different rootstocks was calculated using the following equation: $S(X_i) = (X_i - X_{imin})/(X_{imax} - X_{imin})$ (i = 1, 2, 3, ..., n), where S (X_i) is the subordinate function value of the ith trait of the grafted seedlings on each rootstock, X_{imin} and X_{imax} are the minimum

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and maximum values of the i^{th} trait for all grafted seedlings, respectively. When the value of the trait was negative correlated with the seedling quality, S was determined by the replacement equation $S(X_i) = 1 - (X_i - X_{imin})/(X_{imax} - X_{imin})$. Seedling quality of 'Xiangling' engrafted with different rootstocks was graded according to the mean value of S for all the studied traits.

RESULTS AND DISCUSSION

Growth. Growth is one of the most fundamental processes of plants, growth performance indexes such as PH, BD, TLA, and TDB are always been used as effective and important indicators of plant vitality (Liu et al. 2012, 2020; Shabbir et al. 2021; Thapa et al. 2021; Mostakhdemi et al. 2022). Germplasm resources of different genotypes (species or variety) always exhibit different degrees of phenotypic variation under the same condition (Norouzi et al. 2017; Khadivi et al. 2018; Zarei et al. 2019; Hashemi, Khadivi 2020; Arab et al. 2022). Rootstock is widely used for vegetative propagation of woody perennial fruit trees (such as *Malus*, *Citrus*, *Juglans*, *Prunus*, etc.) mainly due to its considerable important effect on the scion vegetative growth (Warschefsky et al. 2016; Baron et al. 2019; Vahdati et al. 2021). In the present study, walnut rootstock choice showed great influence on scion growth as manifested by the high CV in PH (39.848%), BD (9.190%), TDB (23.665%), TLA (21.428%), and RSR (16.899%) of the scion (Table 2). Compared with 'Xiangling' with *JM* and *JH* as rootstock, the ones grafted on *JRJ*, *JRX*, and *JRL* had higher PH, BD, TDB, TLA, and RSR suggesting higher vitality and vigour. All the growth performance indicates that *JRJ*, *JRX*, and *JRL* would be the better choice for rootstock under the normal environmental condition. The result is consistent with those of our previous study on the seedling growth performance of six walnut rootstock species which indicated that the germplasm variation was mainly attribute to the inherent genetic characteristics of the species (Liu et al. 2020; Arab et al. 2022). The beneficial effects of rootstock on growth performance of the variety mainly due to the well-developed root system with deeper lateral branching which improved the absorbing ability of water and nutrition (Albacete et al. 2015; Warschefsky et al. 2016; Jerszurki et al. 2017; Rasool et al. 2020; Verdugo-Vásquez et al. 2021; Vahdati et al. 2021). However, it is worth noting that scions also have ubiquitous and important effect on modifying phenotype of the rootstocks by providing carbohydrate, hormones, and nucleic acids for the root system that has substantial effects on root phenology and growth (Gaion, Carvalho 2017; Peccoux et al. 2018; Gaut et al. 2019; Gautier et al. 2019; Vahdati et al. 2021).

Gas exchanges. Photosynthesis is the basal mechanism in plant metabolism, it plays a critical role

Table 2. Growth performance of 'Xiangling' walnut grafted onto different rootstock species

Rootstock	PH (cm)	BD (mm)	TDB (g)	TLA (dm^2)	RSR
JRJ	31.41 ± 11.03^{a}	7.20 ± 1.12^{a}	57.40 ± 2.42^{a}	18.30 ± 2.77^{ab}	1.32 ± 0.06^{a}
JRX	35.38 ± 13.96^{a}	7.49 ± 0.77^{a}	58.84 ± 2.45^{a}	20.10 ± 2.34^{a}	1.20 ± 0.06^{a}
JRL	35.61 ± 15.27^{a}	7.92 ± 0.86^{a}	50.77 ± 2.34^{ab}	14.21 ± 1.50^{bc}	1.11 ± 0.23^{ab}
JM	19.00 ± 4.67^{b}	5.99 ± 0.31^{b}	32.23 ± 3.36^{c}	12.92 ±0.46^{c}	0.94 ± 0.10^{ab}
JH	15.38 ± 5.63^{b}	6.86 ± 0.89^{ab}	35.35 ± 3.39^{c}	11.37 ± 1.40^{c}	0.81 ± 0.07^{b}
Mean	30.91	7.09	46.92	15.38	1.08
SD	12.314	0.652	11.103	3.296	0.182
CV (%)	39.843	9.190	23.665	21.428	16.899
F_{RS}	12.904**	1.882	30.909***	6.008*	3.807

JRJ – *Juglans regia* L. cv. 'Jizhaomian'; *JRX* – *Juglans regia* L. cv. 'Xiangling'; *JRL* – *Juglans regia* L. cv. 'Lvxiang'; *JM* – *Juglans mandshurica*, Maxim.; *JH* – *Juglans hopeiensis* Hu.; PH – plant height; BD – basal diameter; TDB – total dry biomass; TLA – total leaf area; RSR – root/shoot ratio; SD – standard deviation; CV – coefficient of variation; F_{RS} – effect of rootstock species
Values are means of five replicates ± SD

$^{a–c}$ Values are mean of all replicates for the same parameter of the 'Xiangling' walnut grafted onto five different rootstock species
Lowercases in the same column show statistically significant differences among 'Xiangling' walnut grafted onto different rootstocks for the same parameter at $P \leq 0.05$ based on Duncan's means tests

*,**, *** indicate significant at $P \leq 0.05$, 0.01, and 0.001, respectively

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in plants' vegetative and reproductive growth. Generally, seedlings of different species or varieties always exhibit a degree of difference in the function of the photosynthetic system, which was directly influenced by the leaf anatomical structure and physical characteristics of the specific species or variety (Liu et al. 2012, 2020; Arab et al. 2022). Under the increasingly serious global environmental change conditions (such as drought, soil salinity, etc.), grafting commercial varieties onto rootstocks with high tress tolerance has been proved to be a cheap and easy technique to improve crop photosynthetic performance and thereby high crop productivity (Schwarz et al. 2010; Kumar et al. 2017; Fullana-Pericàs et al. 2019; Vahdati et al. 2021; Arab et al. 2022). SPAD, reflecting leaf relative chlorophyll content and indicating the health of the plants, always been used as an effective and important indicator of photosynthetic photosynthetic potential and hence primary production (Xiong et al. 2015). According to the one-way ANOVA in Table 3, rootstock selection had significant effect on SPAD ($P \le 0.01$), P_n ($P \le 0.05$), g_s ($P \le 0.01$), and T_r ($P \le 0.001$) of 'Xiangling'. CV of the four parameters was greater than 10.00% suggesting a large variation in photosynthetic capacity among 'Xiangling' engrafted with different rootstock species. Seedlings grafted onto *JRJ* (13.31μmol CO_2/m/s) showed the highest P_n, which was significantly ($P \le 0.05$) higher than those with *JH* (9.20 μmol CO_2/m/s) as rootstock and not significantly ($P > 0.05$) different from those grafted onto *JRX* (11.63 μmol CO_2/m/s), *JRL* (11.24 μmol CO_2/m/s), and *JM* (10.68 μmol CO_2/m/s). SPAD in leaves of 'Xiangling' grafted onto *JRJ* (37.68), *JRX* (37.02), *JRL* (38.26), and *JH* (38.14) was significantly ($P \le 0.05$) higher than those of 'Xiangling' with *JM* as rootstock (29.10). The better growth performance of *JRJ*, *JRX*, and *JRL* was confirmed again by the higher value in P_n and SPAD, which suggesting the potential choice of rootstock under normal condition. Scion gas exchange parameters are controlled genetically by the rootstock through different genetic architectures (Gauthier, Jacobs 2011; Marguerit et al. 2012; Vahdati et al. 2021). Besides the influence of rootstock selection on gas exchanges under normal condition, rootstock had much greater effect on gas exchanges under stress conditions (Kumar et al. 2017; Fullana-Pericàs et al. 2019; Frioni et al. 2020; Vahdati et al. 2021; Arab et al. 2022). Higher leaf chlorophyll and better scion growth performance enabled by a particular rootstock related with higher root hydraulic conductance, deeper root elongation, and higher water and nutrient acquisition capacity resulted in higher photosynthetic capacity and more accumulation of photoassimilates and then resulted in the final higher biomass (Jerszurki et al. 2017; Kumar et al. 2017; Fullana-Pericàs et al. 2019; Cháves-Gómez et al. 2020; Faria-Silva et al. 2020; Vahdati et al. 2021).

Table 3. Gas exchanges of 'Xiangling' walnut grafted onto different rootstock species

Rootstock	SPAD	P_n (μmol CO_2/m/s)	g_s (mol H_2O/m/s)	C_i (μmol CO_2/mol)	T_r (mmol H_2O/m/s)
JRJ	37.68 ± 1.90^{a}	13.31 ± 0.55^{a}	0.51 ± 0.12^{b}	243.14 ± 18.79^{a}	2.74 ± 0.23^{a}
JRX	37.02 ± 2.07^{a}	11.63 ± 1.71^{ab}	0.61 ± 0.13^{ab}	236.50 ± 29.87^{a}	2.36 ± 0.44^{b}
JRL	38.26 ± 2.47^{a}	11.24 ± 2.67^{ab}	0.50 ± 0.23^{b}	214.78±16.00^{a}	2.77 ± 0.13^{a}
JM	29.10 ± 2.76^{b}	10.68 ± 2.14^{ab}	0.70 ± 0.12^{a}	241.08 ± 21.46^{a}	2.76 ± 0.10^{a}
JH	38.14 ± 1.37^{a}	9.20 ± 1.25^{b}	0.43 ± 0.04^{b}	226.82 ± 8.37^{a}	2.39 ± 0.10^{b}
Mean	36.04	11.21	0.55	232.46	2.24
SD	6.651	1.335	0.09	10.479	0.285
CV (%)	18.454	11.906	17.172	4.508	12.718
F_{RS}	10.805**	7.144*	12.860**	0.446	21.671***

JRJ Juglans regia L. cv. 'Jizhaomian'; *JRX – Juglans regia* L. cv. 'Xiangling'; *JRL – Juglans regia* L. cv. 'Lvxiang'; *JM – Juglans mandshurica*, Maxim.; *JH – Juglans hopeiensis* Hu.; SPAD – leaf chlorophyll index; P_n – photosynthetic rate; C_i – intercellular CO_2 concentration; g_s – stomatal conductance; T_r – transpiration rate; SD – standard deviation; CV – coefficient of variation; F_{RS} – effect of rootstock species

Values are means of five replicates ± SD

$^{a-b}$ Values are mean of all replicates for the same parameter of the 'Xiangling' walnut grafted onto five different rootstock species

Lowercases in the same column show statistically significant differences among 'Xiangling' walnut grafted onto different rootstocks for the same parameter at $P \le 0.05$ based on Duncan's means tests

$^{*, **, ***}$ indicate significant at $P \le 0.05$, 0.01, and 0.001, respectively

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Chlorophyll fluorescence. Chlorophyll fluorescence has been widely used as a fast, non-destructive and relatively simple technique to detect leaf physiological status and photosynthetic capacity. It not only reflects the primary reaction of photosynthesis such as absorption of light, transfer of excitation energy, and photochemical reaction, but also is related to electron transport, establishment of proton gradient, ATP synthesis, and CO_2 fixation (Murchie, Lawson 2013; Sitko et al. 2017). No significant ($P > 0.05$) differences in the chlorophyll fluorescence parameters including F_v/F_m, F_v'/F_m', *qP*, and ΦPSII were observed among 'Xiangling' engrafted with the five different rootstock species (Table 4). CV of the studied chlorophyll fluorescence parameters ranged from 1.15% to 14.532% suggesting a small variation among the engrafted plants with different rootstock species. F_v/F_m and F_v'/F_m' for 'Xiangling' grafted onto the five rootstocks ranged from 0.83 to 0.86, and from 0.62 to 0.68, respectively. And no significant ($P > 0.05$) differences in those two parameters were found among the studied engrafted seedlings. It indicated that the choice of rootstock species has no adverse effect on 'Xiangling'. Also, the studied plants were not subject to any stresses including biotic and abiotic factors. Values in *qP* and ΦPSII of 'Xiangling' grafted onto *JRJ* and *JRX* were significantly ($P \leq 0.05$) higher than those with *JM* and *JH* as rootstock, suggesting that *JRJ* and *JRX* helps the grafted seedlings maintain the PSII photochemical efficiency of leaves at a sufficiently high level. *JRJ* and *JRX* would be the better choice for rootstock under environmental stress conditions. Genotyical variation in chlorophyll fluorescence parameters also have been studied to evaluate the crop growth and production under stress conditions (Bresson et al. 2015; Liu et al. 2019; Cháves-Gómez et al. 2020; Verdugo-Vásquez et al. 2021; Arab et al. 2022).

Multivariate analysis. Multivariate statistical techniques which simultaneously analyze multiple variables on each individual, are widely used in analysis of genetic diversity based on morphological, physiological, biochemical or molecular biological traits (Jolliffe, Cadima 2016). Among the multivariate statistical techniques, PCA and HCA are most commonly employed and appear particularly useful for evaluation and characterization of germplasm collections such as *Punica* (Khadivi et al. 2018), *Ziziphus* (Norouzi et al. 2017), *Morus* (Hashemi, Khadivi 2020), *Pyrus* (Zarei et al. 2019), *Juglans* (Liu et al. 2020; Arab et al. 2022), *Mangifera* (Faria-Silva et al. 2020), etc.

PCA reduces the dimensionality of data, transforms the original variables into a few new effective PCs and retains as much as possible of the total variables in only a few PCs. The high correlation between the original variables (Table 5) and the extracted PCs emphasized by this method could help in simplifying evaluation indexes (Jolliffe, Cadi-

Table 4. Chlorophyll fluorescence of 'Xiangling' walnut grafted onto different rootstock species

Rootstock	F_v/F_m	F_v'/F_m'	*qP*	ΦPSII
JRJ	0.85 ± 0.01^{a}	0.68 ± 0.0^{a}	0.42 ± 0.05^{a}	0.29 ± 0.04^{a}
JRX	0.85 ± 0.01^{a}	0.66 ± 0.02^{a}	0.45 ± 0.06^{a}	0.30 ± 0.04^{a}
JRL	0.86 ± 0.00^{a}	0.64 ± 0.01^{a}	0.40 ± 0.05^{a}	0.26 ± 0.03^{ab}
JM	0.83 ± 0.01^{a}	0.62 ± 0.02^{a}	0.33 ± 0.03^{b}	0.20 ± 0.02^{b}
JH	0.85 ± 0.00^{a}	0.64 ± 0.01^{a}	0.36 ± 0.05^{b}	0.23 ± 0.03^{b}
Mean	0.85	0.65	0.39	0.26
SD	0.010	0.020	0.043	0.037
CV (%)	1.155	3.148	10.871	14.532
F_{RS}	2.814	2.102	1.835	3.845

JRJ – Juglans regia L. cv. 'Jizhaomian'; *JRX – Juglans regia* L. cv. 'Xiangling'; *JRL – Juglans regia* L. cv. 'Lvxiang'; *JM – Juglans mandshurica*, Maxim.; *JH – Juglans hopeiensis* Hu.; F_v/F_m – maximal photochemical efficiency; F_v'/F_m' – excitation energy capture efficiency of PSII reaction centers; *qP* – photochemical quenching; ΦPSII – effective PSII quantum yield; SD – standard deviation; CV – coefficient of variation; F_{RS} – effect of rootstock species

Values are means of five replicates ± SD

[a,b] Values are mean of all replicates for the same parameter of the 'Xiangling' walnut grafted onto five different rootstock species

Lowercases in the same column show statistically significant differences among 'Xiangling' walnut grafted onto different rootstocks for the same parameter at $P \leq 0.05$ based on Duncan's means tests

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ma 2016). Results from the PCA in Table 6 indicated that 2 PCs with eigenvalues greater than 1 were extracted and 89.686% of the observed variability can be explained by them. For each factor, characteristics with principal component loading higher than 0.800 were considered as significant. PC_1 was most strongly associated with variables related to growth, biomass accumulation and allocation including PH, TDB, TLA, RSR, and SPAD which represented 61.840% of the total observed variability. The PC_2 accounted for 27.846% of the total variance, and was most strongly associated with eight variables including P_n, g_s, C_i, T_r, F_v/F_m, F_v'/F_m', qP, and ΦPSII. According to the C value in Table 7, 'Xiangling' with *JRJ* as rootstock exhibited the best performance as manifested by the highest C (0.898), followed by the ones grafted onto *JRX* (0.617), *JRL* (–0.150), *JM* (–0.412), and *JH* (–0.953).

HCA, a tool for discovering and identifying associations within the data, divides the studied objects into a few groups (clusters) on the basis of dataset, objects in a cluster are more similar to each other than to objects in other clusters (Köhn, Hubert 2015). Compared with PCA, all the variables in the dataset were used to assemble the clusters in HCA. Therefore, characterizations combined with PCA and HCA could provide more information, which have been widely used in agriculture, horticulture and forestry for germplasm evaluation and screening (Olliffe, Cadima 2016). According to the HCA results from Figure 1, a dendrogram was produced with three main clusters when Euclidean distance was 10. Cluster I included 'Xiangling' engrafted with three rootstock genotypes (*JRJ*, *JRX* and *JRL*) that were normal walnut species and characterized by good growth performance. Cluster II included 'Xiangling' with *JM* as rootstock which was characterized by medium growth performance. 'Xiangling' with *JM* as rootstock was grouped into Cluster III which was characterized by poor growth performance.

Subordinate function analysis. According to the principle of fuzzy mathematics, SFA was widely used for comprehensive evaluation and screening of germplasm resources (Liu et al. 2015; Su et al. 2016). Based on the studied growth, morphological and physiological indicators reflecting plant quality, SFA was carried out to comprehensively evaluate the seedling quality of 'Xiangling' engrafted with five different rootstock species. Seedling quality of 'Xiangling' engrafted with five different rootstocks was graded according to the mean value of S for all the studied traits. SFA in Table 8 indicated that the seedling quality of 'Xiangling' engrafted on the five rootstock species were (from high to low) – *JRJ*, *JRX*, *JRL*, *JM*, and *JH*, which is the same as the

Table 5. Bivariate correlations among the studied parameters of 'Xiangling' walnut grafted onto the five rootstock species

Variables	PH	BD	TDB	TLA	RSR	SPAD	P_n	g_s	C_i	T_r	F_v/F_m	F_v'/F_m'	qP
BD	0.607*												
TDB	0.903***	0.772**											
TLA	0.791**	0.453	0.901***										
RSR	0.975***	0.532*	0.715**	0.887**									
SPAD	0.309	0.758**	0.906***	0.738**	0.301								
P_n	0.448	0.338	0.785**	0.793**	0.266	0.806**							
g_s	–0.107	–0.518*	–0.136	0.217	0.087	–0.041	0.161						
C_i	0.178	–0.588*	0.053	0.419	0.297	–0.129	0.434	0.239					
T_r	0.38	–0.449	0.176	0.488	0.405	0.180	0.454	0.727**	0.276				
F_v/F_m	0.473	0.950***	0.611*	0.232	0.344	0.599*	0.767**	–0.756**	–0.068	–0.633*			
F_v'/F_m'	0.842**	0.516*	0.840**	0.775**	0.807**	0.625*	0.556*	–0.353	0.314	0.239	0.480		
q^P	0.779**	0.781**	0.719**	0.893**	0.806**	0.804**	0.635*	–0.174	0.017	0.056	0.632*	0.810**	
ΦPSII	0.832**	0.761**	0.789**	0.888**	0.840**	0.803**	0.693*	–0.239	0.069	0.091	0.636*	0.886**	0.989***

PH – plant height; BD – basal diameter; TDB – total dry biomass; TLA – total leaf area; RSR – root/shoot ratio; SPAD – soil and plant analyzer development; P_n – photosynthetic rate; C_i – intercellular CO_2 concentration; g_s – stomatal conductance; T_r – transpiration rate; F_v/F_m – maximal photochemical efficiency; F_v'/F_m' – excitation energy capture efficiency of PSII reaction centers; qP – photochemical quenching; ΦPSII – effective PSII quantum yield

*, **, *** indicate significant at $P \leq 0.05$, 0.01, and 0.001, respectively

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Table 6. Eigenvalues, proportion of total variability, as well as correlations between the original variables and the first 2 principal component

Variable	PC_1	PC_2
PH	0.949	0.124
BD	0.736	–0.646
TDB	0.992	–0.044
TLA	0.887	0.335
RSR	0.948	0.272
SPAD	0.912	–0.071
P_n	0.429	0.845
g_s	–0.152	0.803
C_i	0.101	0.914
T_r	0.233	0.963
F_v/F_m	0.587	–0.808
F_v'/F_m'	0.050	0.876
q^P	–0.124	0.937
ΦPSII	–0.103	0.961
Eigenvalue	8.658	3.898
Variance contribution (%)	61.840	27.846
Cumulative variance contribution rate (%)	61.840	89.686

PH – plant height; BD – basal diameter; TDB – total dry biomass; TLA – total leaf area; RSR – root/shoot ratio; SPAD – soil and plant analyzer development; P_n – photosynthetic rate; C_i – intercellular CO_2 – concentration; g_s – stomatal conductance; T_r – transpiration rate; F_v/F_m – maximal photochemical efficiency; F_v'/F_m' – excitation energy capture efficiency of PSII reaction centers; *qP* – photochemical quenching; ΦPSII – effective PSII quantum yield

result from PCA and HCA. It verified the reliability of our results and suggested that the three methods could be used to comprehensively evaluate the seedlings quality of 'Xiangling' engrafted with different walnut rootstock species.

CONCLUSION

In conclusion, the present study revealed that rootstock selection had a considerable effect on phenotype of 'Xiangling' walnut seedlings. PH and SPAD were identified as the most useful indictors for growth evaluation of 'Xiangling' engrafted with five different rootstock species. PCA, HCA and SFA can be used to comprehensively evaluate the quality of engrafted seedlings. Seedling quality of 'Xiangling' engrafted with the five rootstock species from high to low were – *JRJ*, *JRX*, *JRL*, *JM* and *JH*. The present findings demonstrated that *JRJ*, *JRX*, and *JRL* are potential rootstock candidates in the studied walnut planting regions.

Table 7. Values of principal components (*V*) and comprehensive analysis indexes (*C*) of 'Xiangling' walnut grafted onto different rootstock species

Rootstock	*V*		*C*
	V_1	V_2	
JRJ	1.031799	0.60282	0.898257
JRX	0.787629	0.235178	0.616719
JRL	0.285629	–1.11641	–0.14985
JM	–1.14351	1.210255	–0.41247
JH	–0.96154	–0.93184	–0.95265

JRJ – *Juglans regia* L. cv. 'Jizhaomian'; *JRX* – *Juglans regia* L. cv.'Xiangling'; *JRL* – *Juglans regia* L. cv. 'Lvxiang'; *JM* – *Juglans mandshurica* Maxim; *JH* – *Juglans hopeiensis* Hu

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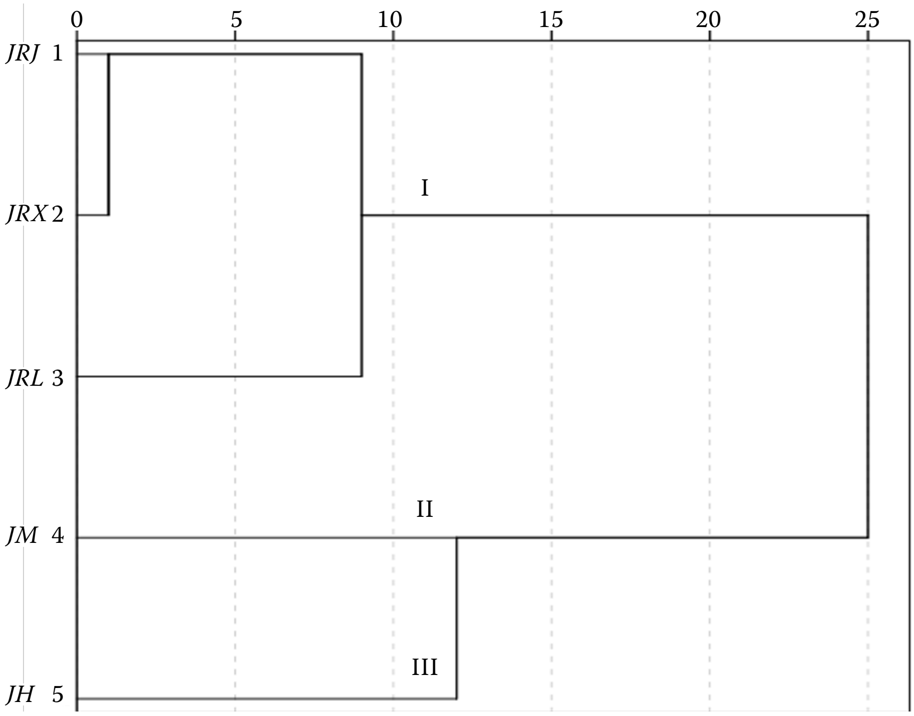

Figure 1. Cluster analysis for the 'Xiangling' walnut grafted onto five different rootstock species based on the quality characteristics
JRJ – *Juglans regia* L. cv. 'Jizhaomian'; *JRX* – *Juglans regia* L. cv. 'Xiangling'; *JRL* – *Juglans regia* L. cv. 'Lvxiang'; *JM* – *Juglans mandshurica* Maxim.; *JH* – *Juglans hopeiensis* Hu.

The results would provide important implication for the selection of proper rootstock species and the breeding of rootstock with specific characteristic. However, researches about the comparative analysis of these rootstock effects on stress tolerance, fruit setting, production and quality

Table 8. Subordinate function values (S) of 'Xiangling' walnut grafted onto different rootstock species

Rootstock	*JRJ*	*JRX*	*JRL*	*JM*	*JH*
PH	1.000	0.592	0.599	0.107	0.000
BD	0.627	0.777	1.000	0.000	0.451
TDB	0.946	1.000	0.697	0.000	0.117
TLA	0.794	1.000	0.325	0.178	0.000
RSR	1.000	0.765	0.588	0.255	0.000
SPAD	0.924	0.837	1.000	0.192	0.000
P_n	1.000	0.591	0.496	0.36	0.000
g_s	0.296	0.667	0.259	1.000	0.000
C_i	1.000	0.767	0.000	0.928	0.425
T_r	0.983	0.658	0.154	1.000	0.000
F_v/F_m	0.667	0.667	1.000	0.000	0.667
F_v'/F_m'	1.000	0.667	0.333	0.000	0.333
qP	0.750	1.000	0.583	0.000	0.250
ΦPSII	0.900	1.000	0.600	0.600	0.600
Mean	0.849	0.785	0.545	0.330	0.203

JRJ – *Juglans regia* L. cv. 'Jizhaomian'; *JRX* – *Juglans regia* L. cv. 'Xiangling'; *JRL* – *Juglans regia* L. cv. 'Lvxiang'; *JM* – *Juglans mandshurica* Maxim.; *JH* – *Juglans hopeiensis* Hu.; PH – plant height; BD – basal diameter; TDB – total dry biomass; TLA – total leaf area; RSR – root/shoot ratio; SPAD – soil and plant analyzer development; P_n – photosynthetic rate; C_i – intercellular CO_2 concentration; g_s – stomatal conductance; T_r – transpiration rate; F_v/F_m – maximal photochemical efficiency; F_v'/F_m' – excitation energy capture efficiency of PSII reaction centres; *qP* – photochemical quenching; ΦPSII – effective PSII quantum yield

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of the grafted trees are urgently needed in our future work.

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