

SCIENTIFIC ARTICLE

Water stress enhances geranium (*Pelargonium*) cuttings rooting quality

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Abstract

Pelargonium nurseries in Europe encounter rooting problems due to the low quality of the cuttings supplied during the winter. The problem may be due to the poor quality of the stock plants from which the cuttings are harvested. The main problem that growers have is the prolonged shipping period and its effect on the viability and rootability of the cuttings once they arrive in Europe. We tested the effect of water stressing the stock plants grown in random block design in a commercial nursery and checked the rootability of cuttings after storage and the susceptibility of cuttings to rot. We assumed that mild water stress could harden the stock plant and thus the cuttings, and they would survive the journey better. Mild water stress improved the rooting ability of some *Pelargonium* varieties after days of storage compared to unstressed plants. Cuttings from water-stressed stock plants showed less decay after prolonged storage than unstressed stock plants as measured by percent rotten cutting. Chlorophyll content increased significantly in cuttings from stressed stock plants. The yield and physical parameters of the cuttings from stressed and nonstressed stock plants did not change. Results indicate that applying water stress to stock plants improves the quality of *Pelargonium* cuttings.

Keywords: *Pelargonium* × *hortorum*, cuttings, rooting, water stress.

Resumo

Estresse hídrico melhora a qualidade das estacas de enraizamento de gerânio (*Pelargonium*)

Os viveiros de *Pelargonium* na Europa enfrentam problemas de enraizamento devido à baixa qualidade das estacas fornecidas durante o inverno. O problema se deve provavelmente à baixa qualidade das plantas matrizes das quais as estacas são colhidas. Concomitantemente, o principal problema que os produtores enfrentam é o período prolongado de envio e seu efeito na viabilidade e enraizamento das mudas quando chegam à Europa. Foi testado o efeito do estresse hídrico nas plantas matrizes cultivadas em blocos aleatórios em um viveiro comercial e verificado o enraizamento das estacas após o armazenamento e a susceptibilidade das estacas ao apodrecimento. A hipótese do trabalho foi que o estresse hídrico moderado poderia enrijecer a planta matriz e, portanto, as estacas sobreviveriam melhor ao transporte. Um baixo estresse hídrico levou a melhora na capacidade de enraizamento de algumas variedades de *Pelargonium*, após dias de armazenamento, em comparação com plantas não sujeitas ao estresse. As estacas de plantas matrizes sujeitas ao estresse hídrico mostraram menor decomposição após armazenamento prolongado em comparação com plantas não sujeitas ao estresse, conforme medido pela porcentagem de cortes podres. O teor de clorofila aumentou significativamente em estacas de plantas matrizes sob estresse hídrico. Não foi observada diferença na produtividade e parâmetros físicos das estacas de plantas matrizes sujeitas e não sujeitas a estressadas estresse hídrico. Os resultados indicam que o estresse hídrico em plantas matrizes melhora a qualidade das estacas de *Pelargonium*.

Palavras chave: *Pelargonium* × *hortorum*, estacas, enraizamento, estresse hídrico.

Introduction

Pelargonium commonly known as Geraniums are important spring greenhouse crops for pot culture and landscape usage worldwide. The worldwide trade in geranium cutting is estimated to value 100 million US Dollars, and Israel growers sell for about 5% of the

market. There is approximately 280 geranium species native mainly to South Africa (Blerot et al., 2016). The commercial “Zonal” group (*Pelargonium* × *hortorum*) and the “ivy” (*Pelargonium peltatum*) are the most common and commercially important groups that are cultivated in Israel both for domestic use and as export to Europe as fresh or rooted cuttings. Millions of geranium cuttings are exported

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from all over the world each year to nurseries during the winter for use as starting material for spring sales of potted geraniums (Jahnke et al., 2018).

Pelargonium are propagated from seeds or cuttings (Brentari et al., 2020). Cuttings allow clonal uniformity and the maintenance of registered varieties for royalties. Thus, to market in early spring rooted and flowering *Pelargonium* plants in pots, nurseries in Europe need a reliable source of cutting during the winter. With its warm climate and highly developed floriculture industry, Israel is part of the European suppliers of *Pelargonium* cuttings. However, because shipping long distances take time, the Israel-produced cuttings have to be hardier than local European cuttings shipped short distances.

Cuttings subjected to slight water deficits before being placed in rooting conditions (e.g., mist) might suffer from a delay or a reduction in rooting response (Murthy and Goldfarb, 2001) or the stress may enhance rooting (Magingo, 2017). However, Lebude et al. (2004) found that slightly reducing the water potential of loblolly pine cuttings before root propagation increased their rooting percentage. This finding suggested the possibility of using water stress as a way to harden *Pelargonium* cuttings.

It was also found that water and salinity stress produces alterations in the cell wall of plant cells (Chazen and Neumann, 1994; Neumann, 1995; Cramer et al., 2001). We hypothesized that alterations in cell wall properties resulting from water stress would make the cutting from stressed stock plants more tolerant against pathogens and thus more resilient. Before harvesting, a brief water stress maximized rose-scented geranium essential oil yield (Eiasu et al., 2008). The period preceding pre-cutting is labeled as stock plant, and the subsequent post-rooting period is two periods that were often neglected in the literature concerned with the effects of environmental conditions on adventitious rooting of cuttings (Andersen, 1986).

Therefore, we tested the hypothesis that growing *Pelargonium* stock plants under conditions of mild water stress will produce hardier cuttings that will sustain a prolonged shipping period. We also tested the possibility that mild water-stressing in the stock plants improves cuttings' rooting ability after prolonged cold storage.

Materials and Methods

The upright type Corsa (*Pelargonium* × *hortorum* "Zonal") and the cascading type Lachs-Cascade (*Pelargonium peltatum*) *Pelargonium* varieties were used in this study. The plants were grown in five-liter bags that contained 70% ground volcanic rock (tuff) and 30% peat. The plants were grown in an industrial nursery (Hadar Nursery in Israel) under a plastic cover, in natural sunlight with a day length of about 10.5 to 11.5 during the growing session (December to February). The plants were grown on tables in complete randomized design blocks in 5 liter substrate with five replications in each block. Each

treatment was repeated five times over the growing season.

Both *Pelargonium*-type plants were watered every four days (watered) or six days (stressed) with five liters of water and complimented with standard commercial NPK solution once in two weeks (Kolbo-Lagan, Nehalim, Israel). Five to seven cm long cuttings with a single leaf and a single axillary bud were removed from all plants including terminal buds. Cutting were removed every week for five weeks during the harvest season and storage immediately in cold storage (4 °C).

Measurement of water usage by the plants

Electronic balances with data loggers were used to monitor the water loss from stock plants. Four pots, each with two plants, were placed on a single balance, and the weight of the pots was measured every minute. Watering was always done in the afternoon. Data recording was done every 10 minutes after averaging ten separate one-minute measurements. Thus, 144 consecutive measurements were recorded twenty-four hours, averaging 1440 separate water loss measurements from the plants. Evapotranspiration rate was calculated as the difference in pot's weight between measurements and was normalized per hourly rate. The potted stock plants were stressed for water two weeks before the start of harvesting cuttings.

Treatment of cuttings

Cuttings were severed from the stock plants by a sterile knife and were weighed and measured for length of cutting and leaf, number of leaves, and number of internodes. After the measurement, the cuttings were stored at 4 °C for periods of 1 to 9 days and then transferred to rooting. Cuttings were planted in a rooting mixture of peat and Styrofoam 1:1 and placed on a rooting table with base heating of 22 °C day and night. High humidity was kept by spraying water for 15 seconds 4 times an hour. The percent of rooted cuttings and percent of rotted cuttings was determined after 21 days.

Chlorophyll and protein determination

Whole cuttings were ground in liquid nitrogen to a fine powder. Chlorophyll was extracted determined as published (Merchuk-Ovnat et al., 2019; 2020). Total protein was extracted in whole cuttings by boiling for 20 minutes in water and determined using the Bio-Rad protein kit (Reuveni et al., 1999).

Statistical analysis

Analyses of variance (ANOVA), was performed with the JMP Statistical Discovery Software (SAS Institute Inc., Cary, NC, USA). The ANOVA models included effects related to genotype. The Tukey-Kramer honestly significant difference (HSD) test calculated the differences among means for three or more treatments and the T-test for two treatments (Subban et al., 2020; Kutsher et al., 2021). Significant difference were only at $p < 0.05$ and below.

Results

Water status of the plants

Watering geranium plants in the afternoon did not induce water usage until the next day (Figure 1A). Evapotranspiration rate declined steadily during the days

after watering, as shown in the decrease in pot weight in both plant types (Figure 1A). There was no difference between the two plant types. Upon rewatering at day six, the stock plants regained their transpiration rate (Figure 1B). The decline in evapotranspiration rate under water stress indicates stomatal closure, a classical response to water stress.

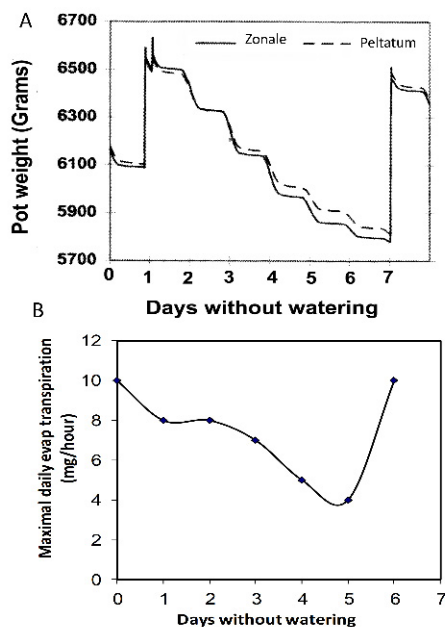


Figure 1. The change in plant and pot weight during a six-day water withholding period. The plant's weight was monitored constantly during the examination as described in methods. **B.** The maximal evapotranspiration rate from both plants' plants is plotted as a function of days of water withholding. The jump in the evapotranspiration rate indicates the second watering after six days.

Following a watering episode, water usage did not change much during the first three days and declined sharply (Figure 1B). After the sixth day, the plants did not extract water from the potting mixture anymore, and the weight of the pots and plants was not changed much (Figure 1A).

Effect of water stress on rooting

The above results calculated the commercial plants' periods and induced mild water stress. Unstressed plants

were watered every four days while still extracting moisture from the root zone (Figures 1A and 1B). Stressed plants were watered every six days when evapotranspiration declined for at least two days. The stressed stock plants showed a 30% reduction in cuttings yield over the five-week water stress period compared to the well-watered stock plants (Figure 2). There was no difference in rooting ability between the two water treatments when cuttings were rooted immediately after harvest.

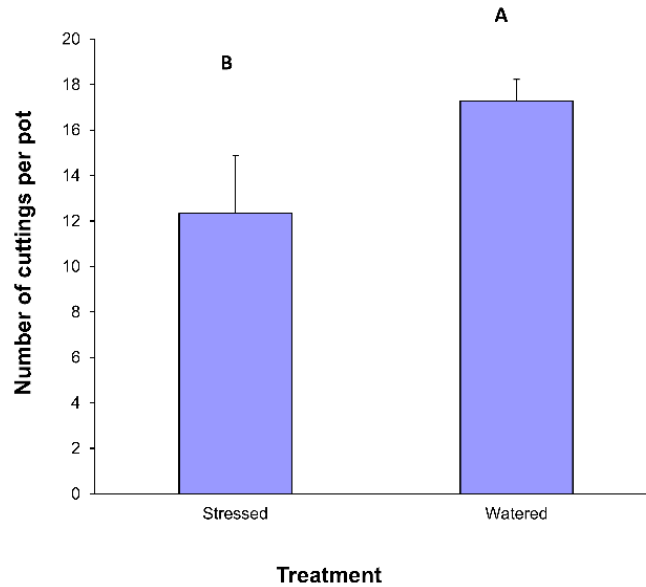


Figure 2. Comparison of cutting yield per pot in both types of *Pelargonium* plants as a function of mild water stress. Student t-test ($p = 0.00026$) was performed in the comparison. Different letters indicate that the averages are significantly distinct above 0.01.

The physical parameters of the cuttings removed from watered and stressed stock plants did not differ significantly upon treatment (Table 1). The lack of difference in those physical parameters indicated that the water stress was not severely affecting stock plant growth.

We tested many cuttings (several hundred altogether) from the commercial nursery and the phytotron. While the stressed plants produced fewer cuttings than the well-watered plants, the cuttings from stressed stock plants did not show statistically significant differences in size or physical parameters except for being darker green.

Effect of water stress on chlorophyll and protein levels

Changes in total soluble protein and chlorophyll in cuttings immediately after removal from the stock plants were recorded as a function of the water status and as markers for stress. There was no difference in protein amounts between cuttings from stressed and watered stock plants ($p = 0.34$ by student t-test). There is a statistically significant difference in the amount of chlorophyll between cuttings from the two treatments ($p = 0.016$ by student t-test). Cuttings from stressed stock plants had 45% higher chlorophyll concentrations than watered plants' cuttings (Figure 3).

Table 1. Effect of water stress on physical parameters of geranium cuttings. Cutting from stressed or well-watered stock plants was tested for growth parameters. Results are an average of 5 replicates, and in each replication, 20 cuttings were measured \pm SE. The same letter indicates that the averages are not significantly different. No significant differences were observed between the treatment of the same plant type.

Treatment	Peltatum		Zonale	
	Watered	Stressed	Watered	Stressed
Leaf length (mm)	46.9 \pm 2.1a	49.1 \pm 0.7a	68.6 \pm 12.4b	67.6 \pm 13.8b
Cutting length (mm)	45.2 \pm 12.9d	43.1 \pm 9.2d	24.3 \pm 5.6e	24.6 \pm 6.9e
Number of internodes	2.6 \pm 0.3g	2.6 \pm 0.1g	4.8 \pm 1.4h	4.7 \pm 1.1h
Cutting weight (gram)	0.7 \pm 0.2i	0.7 \pm 0.2i	2.0 \pm 0.4j	1.9 \pm 0.3j

Each row was tested separately and different letters indicate significance of $p < 0.001$.

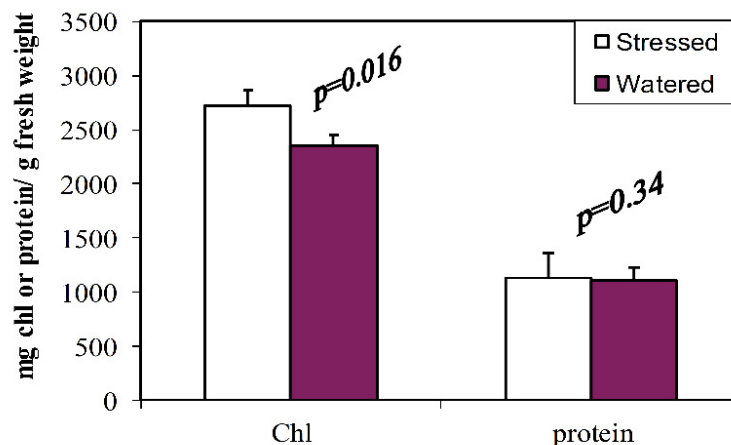


Figure 3. Determination of chlorophyll and total protein in cuttings from Zonal-type *Pelargonium* as a function of the watering status of stock plants. Cuttings were frozen immediately after harvest to measure chlorophyll and protein content as in Methods. Results are an average of 5 replicates, and in each replication, six cuttings were measured \pm SD. Student t-test $p=0.016$ for chlorophyll (Chl) and $p = 0.34$ for protein has performed the comparison. Different letters indicate that the averages are significantly distinct.

Effect of water stressing stock plants on cutting rooting and decomposing

The effect of stress may manifest itself in older stock plants; however, the commercial practice is to replace stock plants every year to decrease the possibility of viral and bacterial contaminations should prevent the deterioration in the stock plants or cuttings yield. We tested the effect of water-stressing stock plant has on cold storage of the cuttings.

There is a clear difference between the two types of geranium genotypes. The cuttings removed from the *Peltatum* variety plants with the harder stems were not affected by water stress. The rooting of cuttings from *Peltatum* is marginally affected by prolonged storage

periods (Figure 4A). Water stressing the stock plants did not improve the rooting behavior of the cuttings (Figure 4A). However, when cuttings were removed from the Zonal-type plant with its soft stems, water stress improved the rooting ability of the cuttings after prolonged storage (Figure 4A). Cuttings from well-watered or stressed stock plants stored at 4 °C up to 4 days showed no difference in rooting ability. More extended storage periods of cuttings removed from the Zonal-type plants reduced the percentage of rooted cuttings from well-watered stock plants (Figure 4A). The cutting originating from water-stressed stock plants did not lose rooting ability even after nine days of storage. Cuttings from well-watered showed only 50% rooting ability (Figure 4A).

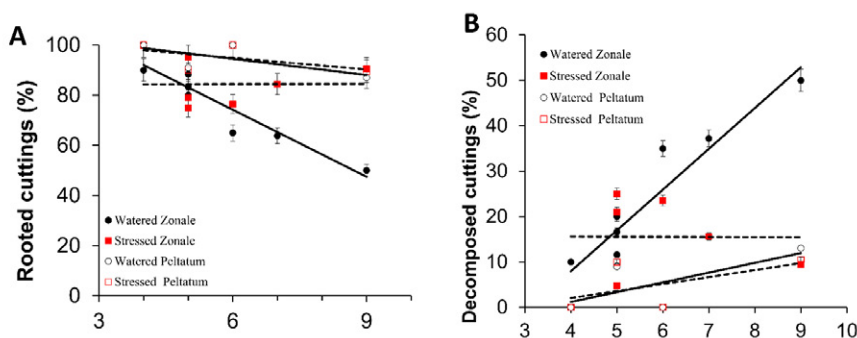


Figure 4. Determination of decomposing and rooting percentage of cuttings as a function of the watering status of stock plants. **A:** Percent of rooting as a function of the stock plants' watering and the cuttings' storage time. Rooting is expressed as the percent of cuttings that are rooted without storage. Rooting was determined after 16 days in the rooting mix. Results are an average of 5 replicates, and in each replication, 20 cuttings were measured. Complete lines show data from watered plants and dashed lines depict data from stressed plants, filled symbols (l, n) Zonale \pm SD, and empty symbols (p, m) *Peltatum* \pm SD. **B:** Percent of decomposing cuttings was determined after 21 days in the rooting mix. Results are an average of 5 replicates, and in each replication, 20 cuttings were measured. Complete lines show data from watered plants and dashed lines depict data from stressed plants, filled symbols (l, n) Zonale \pm SD, and empty symbols (p, m) *Peltatum* \pm SD.

When placed in the rooting mixture on the rooting table (Figure 4B), the cascading variety *Peltatum*-type cuttings were again unaffected by water stress in any way. Both rooting ability and percentage of decomposed *Peltatum*-type cuttings were not improved or harmed by water stressing the stock plants (Figure 4).

There was no noticeable difference in the physical attributes of the cuttings from the zonal variety after nine days of storage at 4 °C. However, there was a notable difference in the percentage of decomposed cuttings during the rooting period between cuttings that originated from well-watered or stressed stock plants (Figure 4B). There is a progressive increase in decomposing of the cuttings removed from the well-watered zonal type geranium variety (Figure 4B). Water stressing the zonal type geranium variety decreased the decomposing of cuttings on the rooting mixture. This finding is significant to cuttings producing nurseries because it shows that mild water stressing the stock plants can produce hardier and less vulnerable cuttings.

We hypothesized that mild water stress of stock plants would produce cuttings that are hardier and of better quality than nonstressed cuttings without affecting yield. Water was withheld from the stock plants, and cuttings were regularly harvested for five weeks tested for visual phenotype and rooting ability. We found that the mild water stress preserved the ability of cuttings to root after more extended storage periods compared to cuttings that were removed from nonstressed stock plants. Of the two types of geranium tested, the cascading type *Peltatum* was much less susceptible to the decline in rooting ability after prolonged storage. As such, mild water stress had a very mild improvement on rooting ability.

Discussion

Drought is a severe environmental stress for non-irrigated crops such as cereals (Havrlentova et al., 2021). Drought stress affects yield and plant performance by reducing yield and decreasing plant growth (Lamaoui et al., 2018; Hussain et al., 2019). The physiological and morphological responses of shoots to drought stress are widely documented. However, the root systems responses were not given much attention due to the lack of straightforward, efficient methods for studying root systems in irrigated soil and also to the fact that in irrigated soils, drought is considered a non-issue. The root system is the main organ that plants have for water and nutrient uptake and thus influences plant growth and productivity (Balliu et al., 2021).

Cuttings have no taproots, and the main structural components of their root systems are adventitious roots formed when placed in media with high humidity. New roots are formed post-embryonically from cells of non-root tissues (Druege et al., 2019). Adventitious roots formation is observed in excised plant parts. Excision-induced adventitious roots formation is a bottleneck for plant survival during vegetative propagation. The development

of the new root system in isolated plant fragments restores competence for water and nutrient uptake, and depends on the cutting present environmental conditions and past physiological status (Druege, 2020). Plants of *Callistemon citrinus* under mild water deficit conditions increased the ratio between photosynthesis and stomatal conductance, producing small but qualitative with reduced water losses and inflorescences similar to the control plants (Giordano et al., 2021).

The results above indicate that mild stress can improve the quality of geranium cuttings by producing hardier cuttings. Similar findings were found for loblolly pine cuttings that were water-stressed before placing in the rooting mixture (Lebude et al., 2004). Generally, the physiological and phenological conditions of the stock plants affect the ability of cuttings to root and survive biotic stress such as storage and rooting media (Magingo 2017; Monder 2018; Murphy 2021). Reducing the yellowing effect of stressed cutting can be achieved by phytohormones treatment (Toscano et al., 2018), but it is expensive and time-consuming. Water stressing the stock plants as a technique for prehardening cuttings to produce a better product may apply to other plants exported as fresh unrooted cuttings. The alterations observed after water and salinity stresses in the cell wall of plants (Chazen and Neumann, 1994; Neumann, 1995; Cramer et al., 2001) may be universal and are responsible for the hardening effect that we observed in geranium cuttings.

Many biotic and abiotic stresses cause a similar increased in defense mechanism genes to cope with these stresses such as Pathogenesis Response (PR) proteins. For example, osmotin, a PR-5 family protein was initially discovered as a water stress responsive protein but turned out to be produced in response to diseases as well. Osmotin was shown to affect the permeability of fungal hyphae (Abed et al., 1996) and inhibit the activity of defensive cell wall barriers and increases its cytotoxic efficiency (Abid Ullah et al., 2018). This mechanism involves cell wall binding and membrane perturbation (Abed et al., 1996). The accumulation of PR proteins in the stock plants as a result of the mild water stress can explain the relative fungal resilience of harvested cuttings.

Conclusions

Mild water stress enhanced the rooting ability of some *Pelargonium* varieties.

Mild water stress does not affect cuttings yield in some *Pelargonium* varieties.

Chlorophyll content increased in cuttings from water-stressed stock.

Author contribution

MR: conceived, designed the experiments, and drafted the paper; **YK, DA:** performed the experiments. All authors critically read and approved the final version of the manuscript.

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