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High-altitude accessions of *Capsicum baccatum* and *C. pubescens* as rootstocks to enhance suboptimal-temperature tolerance of pepper

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ABSTRACT

This study evaluated the effectiveness of rootstocks germplasm to enhance the chilling tolerance of *Capsicum annum* L. We compared 2 accessions of *C. baccatum*, i.e., CB 5 and CB 9, and 3 accessions of *C. pubescens*, i.e., CP 2, CP 3, and CP 4, together with commercial controls across 3 experiments. Experiment 1 (Exp 1) aimed to evaluate these genotypes adaptation compared to rootstock cv. 'Capsifort' to chilling temperatures and was performed in growth chambers for 28 days under two thermal regimes, i.e. optimal (25–15 °C for 12 h/day) and suboptimal (15–5 °C for 12 h/day). In Exp 2 the scion cv. Tiberio was grafted onto these rootstocks and grown under suboptimal temperature for 28 days in comparison to ungrafted control. Exp 3 evaluated the same graft combinations in a commercial greenhouse, to assess the effects of grafting on fruit yield and quality of the scion 'Tiberio' compared to ungrafted and self-grafted controls. In Exp 1, CP 3 showed the best photosynthetic performances, whereas CP 2 and CP 3 outperformed 'Capsifort' for plant biomass under suboptimal temperature. In Exp 2, chlorophyll fluorescence was higher in the combination 'Tiberio'/CB 9 than in 'Tiberio', while 'Tiberio'/CP 3 had a higher photosynthetic rate. The stomatal conductance was higher in 'Tiberio'/CP 3 and 'Tiberio'/CP 4 than in 'Tiberio'. All the graft combinations reduced the leaf proline content compared to the ungrafted plants. In Exp 3 the graft combinations 'Tiberio'/'Capsifort', 'Tiberio'/CB 5, 'Tiberio'/CB 9 and 'Tiberio'/CP 3 produced the highest marketable yields, but 'Tiberio'/'Capsifort', 'Tiberio'/CB 5, and 'Tiberio'/CB 9 had the highest number of discarded fruits per plant. 'Tiberio'/CP 2 fruits had lower titratable acidity values than 'Tiberio', whereas 'Tiberio'/CB 9 had the highest antioxidant activity. Overall, our results showed that CB 9 and CP 3 consistently proved positive performance on 'Tiberio', while CP 2 performed well under low temperature conditions only.

1. Introduction

Low temperatures represent an important environmental stressor affecting growth and productivity of crops globally (Ding et al., 2020). Indeed, suboptimal temperature can generate a reduction in plant growth, metabolic function, cellular integrity, or tissue injury that limits the yield potential. At morpho-physiochemical level, these disturbances can negatively affect both the vegetative and reproductive stages (Nurhasanah Ritonga and Chen, 2020), including reduced seed germination, stunted seedling growth, leaf yellowing, impairment of the photosynthetic apparatus, and reduced rates of photosynthesis. Upon the reproductive stage, cold stress often results in pollen sterility and alterations in ovary development (ovary swelling), leading to the production of misshapen fruits (Aslam et al., 2022; Nurhasanah Ritonga and Chen, 2020). The extent of plant damage resulting from the exposure to low temperatures is species-specific and dependent on the duration and intensity of the stress. Plants originating from warm-climates often experience physiological disturbances even when exposed to suboptimal, non-freezing temperatures (i.e., chilling temperatures) and may not be able to recover entirely (Manasa et al., 2022).

Pepper (*Capsicum annum* L.) is one of the most widely cultivated fruit vegetables worldwide, owing also to its nutraceutical properties (Giuffrida and Leonardi, 2012). This thermophilic species originates from tropical regions of Central and South America, and the optimum growth conditions occur with average daily temperature ranging between 21 and 23 °C (Tripodi and Kumar, 2019). Pepper cultivation in South Italy typically involves transplanting in unheated greenhouses during cold months (from October to January), so plants exposure to suboptimal temperatures is common. Despite being generally cold-sensitive, the *Capsicum* genus comprises several species originating from regions with

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varying altitudes and average temperatures. This biodiversity represents an opportunity for research to focus on enhancing pepper's tolerance to chilling stress. Among these species, *C. pubescens* Ruiz & Pav. and *C. baccatum* L. have been identified as potential candidates in pepper breeding programs (Ou et al., 2015). Indeed, breeding companies constantly strive to develop cultivars with improved tolerance to the environmental stressors. However, this can be a time-consuming process that may not lead to desirable outcomes in the short term, particularly for abiotic stress tolerances, which are often regulated by multiple genes and complex metabolic interactions (Kyriacou et al., 2017). Additionally, high-quality traits may be exhibited by genotypes highly susceptible to the environmental stressors. In such cases, vegetable grafting of elite cultivars onto rootstocks with improved tolerances can provide an effective alternative to the relatively slow breeding programs of the scion cultivars (Mauro et al., 2020b; Ntatsi et al., 2017).

Interspecific grafting is very common within solanaceous species (e.g. Abdelmageed and Gruda 2009, Pico et al. 2017) for improving tolerance to abiotic and biotic stresses. Compared to tomato and eggplant, the selection of available rootstocks for pepper is scarce. Nevertheless, grafting compatibility with *C. baccatum* and *C. pubescens* has been reported (Palada and Wu, 2008; Penella et al., 2017). High-altitude accessions of related *Capsicum* species, with large thermal excursions during the day, often display greater tolerance to low-temperature stress (Venema et al., 2005). In tomato, grafting onto a high-altitude accession of *Solanum habrochaites* L. enabled the plant to overcome vegetative growth reduction under chilling temperatures (Venema et al., 2008). However, the Authors reported significant yield reductions for the grafted plants, likely due to the influence of root signals involved in fruit set (Ntatsi et al., 2014). Hence, the significance of identifying wild accessions as potential rootstocks for enhancing low-temperature tolerance diminishes if increased vegetative growth fails to sustain yield and fruit quality under stress conditions. For these reasons, this study aimed to evaluate the chilling tolerance in growth chamber of five accessions of *C. baccatum* and *C. pubescens* from high altitude regions of South America (Exp 1) and compare them with a widespread pepper rootstock in South Italy. After this step, grafting compatibility with bell pepper was assessed through another growth chamber experiment using 'Tiberio' F_1 as scion, and the chilling tolerance of the graft combinations was evaluated by comparing them with the ungrafted 'Tiberio' F_1 as control (Exp 2). Finally, the same graft combinations of Exp 2 were evaluated in a greenhouse experiment, to assess the effects of these rootstocks on fruit yield and quality of the scion 'Tiberio' F_1 (Exp 3).

2. Materials and methods

Two accessions of *C. baccatum* (CB) and three accessions of *C. pubescens* (CP) were selected from 12 genotypes previously evaluated for their high germination rate and uniformity (data not shown), giving priority to those originating from higher altitude areas (Table 1). The *C. pubescens* accessions PI585267, PI585273, and PI355812 (hereafter CP 2, CP 3, and CP 4, respectively), and the *C. baccatum* accession PI238061 (CB 5), were collected from above 2000 m a.s.l. and have been maintained by the United States Department of Agriculture through the U.S. National Plant Germplasm System (USDA-NPGS). *C. baccatum* 'Bacclaudio' (CB 9) is a local ecotype selected in Northern Italy from high altitude accessions and provided by the USDA-NPGS. The widely used pepper rootstock 'Capsifort' F_1 (Bayer Italia, Milano, Italy) was used as a control (Exp 1). The bell pepper 'Tiberio' F_1 (Vilmorin Mikado Italia, Funo, Italy), which is widely grown in the greenhouse areas of South Italy, was used as scion, to evaluate the chilling tolerance of the graft combinations in growth chamber (Exp 2) and their bio-agronomical performances in a greenhouse experiment (Exp 3).

Table 1
Capsicum spp. accessions used in the preliminary seed germination test; accessions studied in this experiment are indicated in bold (where indicated, information about the site of collection and its elevation were provided from the United States Department of Agriculture – USDA, through U.S. National Plant Germplasm System).

Code	Name	Species	Origin	Elevation
CB 5	**PI238061**	***C. baccatum* var. *baccatum***	**Bolivia, Cochabamba**	**2560 m a.s.l.**
CB 6	PI441659	*C. baccatum* var. *baccatum*	Brasil, Bahia, Vitoria de Conquista	900 m a.s.l.
CB 7	CAP 215	*C. baccatum* var. *baccatum*		
CB 8	Pimenta do passarinho	*C. baccatum* var. *baccatum*		
CB 9	**Bacclaudio**	***C. baccatum* var. *baccatum***		
CB 10	PI596056	*C. baccatum* var. *pendulum*	Bolivia, Chuquisaca	1900 m a.s.l.
CB 11	PI596057	*C. baccatum* var. *pendulum*	Bolivia, Chuquisaca	1870 m a.s.l.
CB 12	PI596058	*C. baccatum* var. *pendulum*	Bolivia, Chuquisaca	2150 m a.s.l.
CP CC 1	Rocopica	*C. pubescens* × *C. cardenasii*		
CP 2	**PI585267**	***C. pubescens***	**Ecuador, Azuay**	**2372 m a.s.l.**
CP 3	**PI585273**	***C. pubescens***	**Ecuador, Carchi**	**2740 m a.s.l.**
CP 4	**PI355812**	***C. pubescens***	**Ecuador, Azuay, San Fernando**	**2800 m a.s.l.**

2.1. Evaluation of high-altitude accessions grown under two thermal regimes in growth chamber (Exp 1)

2.1.1. Growth conditions

The experiment was conducted using two separate growth chambers, each having a volume of 15 m^3, where high sodium pressure lamps were used for providing a white light (500 μmol m^{-2} s^{-1} for 12 h per day). Plants from the 5 selected accessions, i.e., CB 5, CB 9, CP 2, CP 3, and CP 4 and 'Capsifort' F_1, used as control, were grown in each growth chamber, starting from the stage of 2 true leaves. A closed hydroponic system was installed, which included covered black tanks (16 L) filled with a half-strength Hoagland's solution having pH 5.8 and refreshed weekly. Additionally, 2 aeration pumps (Jeneca AP-9800 air pump, 1.6 L min^{-1}) were installed for each tank. A randomized blocks design was used, in which 2 tanks represented an experimental unit with 9 plantlets each, arranged in a rectangular format (0.20 × 0.20 m, 25 plant m^{-2}). To ensure consistent growth conditions, the tanks' position was changed once a day. Two thermal regimes were employed: an optimal regime with a temperature of 25–15 °C (12/12 h day^{-1}) and a suboptimal one with a temperature of 15–5 °C (12/12 h day^{-1}). The suboptimal regime was selected to mimic the microclimate conditions of an unheated greenhouse during winter in the reference area. The experiment lasted for 28 days.

2.1.2. Physiological measurements

Photosystem II efficiency was measured 28 days after the beginning of the growth chamber experiment, through chlorophyll *a* fluorescence analysis using an OS1-FL fluorometer (Opti-Sciences Corporation, Tyngsboro, MA). Chlorophyll fluorescence excitation was performed on the uppermost fully expanded leaves, by using a 660 nm solid-state light source coupled with filters able to block λ above 690 nm; the modulated light intensity was adjusted from 0 to 1 μE. Fluorescence detection was performed between 700 and 750 nm using a PIN silicon photodiode coupled with appropriate filtering to remove extraneous light. Saturation of photosystem II was provided by a filtered 35 W halogen lamp at

350–690 nm, which performed an 800 ms light pulse. All measurements were performed after a 30 min leaf dark-adaptation through OS cuvettes. This allowed for the measurement of the ratio among variable and maximum fluorescence. On the same date and leaves, gas exchange measurements were performed through a LCi Portable Photosynthesis System (ADC BioScientific Ltd., Hoddesdon, UK). Instantaneous net photosynthesis and stomatal conductance were measured by recording duplicate measurements in 9 plants per plot. Measurements were taken with an average air CO_2 concentration inside the growth chamber of ~385 ppm.

Electrolyte leakage of leaves was measured on the same date, soon after removing plants for destructive analysis, according to Mauro et al. (2020a). For each measurement, 20 leaf discs, 1 cm^2 each and taken from the uppermost fully expanded leaves, were collected and placed in a 50 mL tube containing 20 mL of ultrapure water. The tubes were placed in a shaker at 100 rpm for 24 h at 25 °C. A first EC reading (EC1) was performed at the end of the 24 h shaking, then the test tubes were placed in an autoclave at 120 °C for 20 min and left to cool again at 25 °C for the second reading (EC2). Electrolyte leakage was calculated as EC1/EC2 ratio and expressed on a percentage basis.

2.1.3. Plant growth and developmental measurements

Stem diameter at the collar region, plant height, number of leaves and leaf area per plant through an Area Measurement System (Delta-T Devices LTD, Burwell, Cambridge, UK) were measured in plants exposed to both thermal regimes on day 28. Shoot, root and plant dry biomass were determined through the gravimetric method, by using a thermo-ventilated oven at 105 °C, until constant weight of the samples, i.e., after ~72 h.

2.2. Evaluation of *C. annuum* 'Tiberio' F_1 grafted onto high altitude accessions and 'Capsifort' F_1 under low temperature regime (Exp 2)

2.2.1. Plant materials and growth conditions

The scion 'Tiberio' F_1 grafted onto the above-mentioned *C. baccatum* ('Tiberio'/CB 5; 'Tiberio'/CB 9), *C. pubescens* ('Tiberio'/CP 2; 'Tiberio'/CP 3; 'Tiberio'/CP 4), genotypes and 'Capsifort' ('Tiberio'/'Capsifort') together with the ungrafted control were grown in the same hydroponic system as Exp 1, under the suboptimal thermal regime. Plants were obtained from a specialized nursery, where the splice-grafting technique was applied (Bie et al., 2017), using plastic clips and sticks to secure the graft union. After 28 days the experiment was ended, and the measurements reported below were carried out.

2.2.2. Physiological measurements

Chlorophyll *a* fluorescence, net photosynthetic rate, stomatal conductance and electrolyte leakage were determined following the same methodologies reported for Exp 1. Additionally, leaf proline content was measured spectrophotometrically according to Khedr et al. (2003) with minor modifications. Briefly, frozen 0.1 g leaf samples were homogenized in 3 % aqueous sulphosalicylic acid and the residues were removed by centrifugation at 12,000 × *g* for 10 min. An aliquot of supernatant was mixed with 1 mL of glacial acetic acid and ninhydrin reagent in a 1:1 (*v/v*) ratio. The reaction mixture was incubated at 100 °C for 1 h, then the reaction was stopped in an ice bath. After extraction with 2 mL of toluene, the absorbance of the properly separated organic phase was read at 520 nm, using toluene as a blank. Proline concentration was determined from a standard curve using D-proline as standard.

2.2.3. Plant growth and developmental measurements

Plant height, number of leaves per plant, leaf area and dry biomass of shoot, root and whole plant were determined on day 28 as previously described for Exp 1. Specific leaf area was calculated as the ratio among total leaf area and leaf dry weight, expressed on a per plant basis.

2.3. Agronomical evaluation of *C. annuum* 'Tiberio' F_1 grafted onto high altitude accessions under cold Mediterranean greenhouse conditions (Exp 3)

2.3.1. Experimental site and growth conditions

The experiment was conducted in Sicily, Southern Italy (36°59′14.15″ N; 14°21′45.54″ E; 13 m a.s.l.), in a 2560 m^2 greenhouse with a sandy soil and an electrical conductivity of the irrigation water ~750 μS cm^{-1}. The greenhouse hosting the crop had a steel tubular frame and windows along the sides, covered before transplanting with an ethylene-vinyl acetate film 200 μm thick and a white/black polyethylene film 30 μm thick mulching the soil. Bell pepper seedlings were obtained from a specialized nursery, where the splice-grating technique was applied (Bie et al., 2017), followed by the application of plastic clips and sticks to secure the graft union. Before the start of the experiment, plants were selected for homogeneous size and apparent healthy status. The seedlings were transplanted on January 15^{th} 2018 at the stage of three true leaves, adopting a 0.40 × 1.00 m rectangular format (2.5 plants m^{-2}), and trained up to May 27^{th} 2018, i.e., 133 days after transplanting. Once transplanted, all plants were managed according to the same standard commercial practices, receiving 20.3 g N, 15.4 g P_2O_5, 21.3 g K_2O, 27.2 g MgO and 0.4 g Fe per plant. Drip irrigation was performed when the accumulated evapotranspiration outside the greenhouse reached 40 mm, estimated through the Penman–Monteith equation. Wood stakes were installed every 2 m on the row for plants training, linked with polypropylene twine on each side of the plants placed horizontally every 0.3 m. Plants were pruned to four branches and fruits were manually harvested at the commercial ripening stage, which was determined in accordance with local customs, i.e., at the beginning of color turning. During the experiment, the greenhouse climatic conditions were recorded every hour though a HOBO Temperature/Relative Humidity Data Logger (Onset Computer Corporation, Bourne, MA, USA).

2.3.2. Plant material and experimental design

Bell pepper plants 'Tiberio' F_1 either grafted onto 'Capsifort' F_1 ('Tiberio'/'Capsifort'), *C. baccatum* ('Tiberio'/CB 5 and 'Tiberio'/CB 9) and *C. pubescens* ('Tiberio'/CP 2, 'Tiberio'/CP 3 and 'Tiberio'/CP 4) accessions, together with ungrafted and self-grafted plants, both as controls, were cultivated at the center of the abovementioned greenhouse, adopting a one-way randomized blocks design with 4 replicates, each consisting of 15 plants (net of borders).

2.3.3. Yield and related components

After each harvest, all collected fruits were sorted into marketable and unmarketable (misshapen). They were subsequently characterized based on total weight, number of fruits per plant, and average fruit weight.

2.3.4. Fruit quality determination

For the quality analysis, fruits harvested from second bifurcations were considered. The dry matter content of the fruits was determined by gravimetric analysis after complete desiccation in a thermoventilated oven (Binder, Milan, Italy) at 105 °C. To measure total soluble solids and titratable acidity, 5 fruits per plot were homogenized into a puree using a home blender (La Moulinette, Groupe SEB, Écully, France), then centrifuged for 10 min at 1,370 × *g*. The clear juice was used to measure total soluble solids through a digital refractometer (DBX-55, Atago, Tokyo, Japan), expressing °Brix at 20 °C. Titratable acidity, expressed as g L^{-1} citric acid equivalents was determined by titrating a 10 mL aliquot of the juice sample with 0.1 M NaOH to pH 8.2, using phenolphthalein as an indicator until the pink endpoint.

The antioxidant activity of the fruit extract was determined using the 1,1-diphenyl-2-piricridrazile (DPPH) assay, according to Brand-Williams et al. (1995) with minor modifications. Briefly, 0.05 g of previously freeze-dried sample were finely grounded (IKA 011 Basic

Analytical Mill) and mixed with 1.5 mL of a methanol solution (80 %), sonicated for 30 min, and centrifuged for 10 min at 5 °C and 5,000 × *g*. An aliquot (0.01 mL) of supernatant was added to 1.4 mL of a daily prepared DPPH solution 150 μM in methanol:water (95:5), vortexed, and incubated in the dark at room temperature (20 °C) for 30 min, then, the absorbance at 517 nm was spectrophotometrically measured with a Jenway 7315 UV–vis spectrophotometer (Cole-Parmer, Stone, UK). A calibration curve was created measuring the percentage of inhibition of the absorbance at 517 nm of Trolox ((±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid 97 %) and antioxidant activity was expressed as micromoles of Trolox equivalents kg^{-1} of fresh weight.

2.4. Statistical analysis

All data were subjected to Shapiro-Wilk's and Levene's tests, to check for normal distribution and homoscedasticity, respectively, then to one-way or two-way analysis of variance (ANOVA), according to the experimental designs adopted in the experiments. Average values were separated through the Tukey's HSD test (at least for $P \leq 0.05$). All calculations were performed using Excel® version 2016 (Microsoft Corporation, Redmond, WA, USA) and Minitab® version 16.1.1 (Minitab Inc., State College, PA, USA).

2.5. Microclimate conditions inside the greenhouse (Exp 3)

Throughout the experiment, the average temperature steadily rose from 16.8 to 25.1 °C in week 1 and week 19 after transplanting, respectively, while relative humidity demonstrated a relatively stable pattern, ranging between 50.0 and 63.0 % in week 15 and week 6 after transplanting, respectively.

3. Results

3.1. Evaluation of high-altitude accessions grown under two thermal regimes in growth chamber (Exp 1)

3.1.1. Physiological variables

CB 9 displayed higher (+4.7 %) chlorophyll *a* fluorescence values compared to 'Capsifort', CB 5 and CP 4 (Table 2) whereas CP 3 showed a higher Chlorophyll *a* fluorescence compared to 'Capsifort' and CP 4. Notably, CP 3 also exhibited the highest net photosynthesis (12.16 μmol CO_2 m^{-2} s^{-1}) compared to the remaining accessions and 'Capsifort' except to CB 9 (Table 2). CP 2 and CP 4 had higher stomatal conductance values (+116.6 %) compared to 'Capsifort', CB 5 and CB 9 (Table 2). Additionally, 'Capsifort' showed the highest electrolyte leakage (37.4 %), whereas CB 9 exhibited the lowest value (17.7 %) among all the accessions (Table 2).

3.1.2. Plant growth and developmental variables

When the genotype *per se* was concerned, all the *Capsicum baccatum* and *C. pubescens* accessions outperformed 'Capsifort' in terms of growth variables, except for stem diameter and the number of leaves $plant^{-1}$ (Table 3); however, the ANOVA revealed a strong interactive effect among the studied factors. Indeed, while under optimal temperature all the *Capsicum baccatum* and *C. pubescens* genotypes exhibited higher values of plant height (between 60.0 and 72.7 cm), number of leaves (between 73.9 and 116.9, except CB 9), and leaf area (between 1734 and 3122 cm^2 $plant^{-1}$) than 'Capsifort', under suboptimal temperature CB 5 was the least performing rootstock in terms of stem diameter (4.44 mm), plant height (16.4 cm) and leaves $plant^{-1}$ (14.4) (Table 3). On the other hand, under suboptimal temperature higher values than 'Capsifort' were recorded for plant height (between 27.3 and 35.4 cm) in CP 4, CP 2 and CP 3 (Table 3), number of leaves (20.4 $plant^{-1}$, on average) in CP 2 and CP 3 (Table 3) and leaf area (between 399 and 452 cm^2 $plant^{-1}$) considering all the *Capsicum baccatum* and *C. pubescens* genotypes (Table 3). Considering the biomass production under optimal temperature conditions 'Capsifort' showed the lowest values in terms of shoot, root and plant biomass (3.04, 0.49 and 3.54 g DW $plant^{-1}$, respectively) (Table 4). However, under low temperature, CB 5 proved the strongest reductions for these variables, so that it showed the least root biomass (0.42 g $plant^{-1}$), whereas its shoot and plant biomass (1.72 and 2.14 g $plant^{-1}$, respectively) did not differ from those recorded in 'Capsifort' (Table 4). Differently, despite their significant reductions across thermal regimes, under low temperature both CP 2 and CP 3 had still the highest shoot (3.56 g DW $plant^{-1}$, on average), root (1.34 g DW $plant^{-1}$, on average) and plant biomass (4.90 g DW $plant^{-1}$, on average), with CB 9 and CP 4 showing intermediate values for root and plant biomass (on average 0.75 and 4.01 g DW $plant^{-1}$, respectively) (Table 4).

3.2. Evaluation of *C. annuum* 'Tiberio' F_1 grafted onto high altitude accessions and 'Capsifort' F_1 under low temperature regime (Exp 2)

3.2.1. Physiological variables

Concerning chlorophyll *a* fluorescence, only 'Tiberio'/CB 9 displayed a higher value than 'Tiberio' (0.805 vs. 0.778), whereas 'Tiberio'/CP 3 showed a higher net photosynthesis than the ungrafted control, 'Tiberio'/'Capsifort' and 'Tiberio'/CB 5 (by 57 %, on average) (Table 5). The graft combinations 'Tiberio'/CP 3 and 'Tiberio'/CP 4 showed higher stomatal conductance values (0.12 mol H_2O m^{-2} s^{-1}), compared to control, 'Tiberio'/CB 5, 'Tiberio'/CB 9 and 'Tiberio'/CP 2 (0.05 mol H_2O m^{-2} s^{-1}, on average) (Table 5). When electrolyte leakage was concerned, the lowest value was observed in 'Tiberio'/CP 2, whereas this variable peaked in the ungrafted control, 'Tiberio'/CB 5 and 'Tiberio'/CP 4 (26.8 %, on average) (Table 5). The rootstock genotype strongly influenced the leaf proline content as well, as all the graft combinations reduced this variable, by up to 62 % in 'Tiberio'/'Capsifort' compared to the ungrafted plants (Table 5).

3.2.2. Plant growth and developmental variables

Plant growth variables and biomass partitioning were significantly influenced by the rootstock (Table 5). Plant height was higher than control in all the *Capsicum baccatum* and *C. pubescens* graft combinations, reaching 19.9 cm in 'Tiberio'/CB 9, whereas no differences were recorded between control and 'Tiberio'/'Capsifort' (15.3 cm, on

Table 2
Chlorophyll a fluorescence (F_V/F_M), net photosynthetic rate (A) stomatal conductance (gs) and electrolyte leakage (EL) of Capsicum spp. leaves after 28 days of growth under suboptimal temperature regime (15–5 °C for 12 h/day) (Exp 1).

Genotype	F_V/F_M (adimensional)	A (μmol CO_2 m^{-2} s^{-1})	gs (mol H_2O m^{-2} s^{-1})	EL (%)
'Capsifort'	0.765 c	7.27 c	0.09 bc	37.45 a
CB 5	0.775 bc	8.00 bc	0.10 b	20.40 cd
CB 9	0.804 a	9.92 ab	0.08 bc	17.68 d
CP 2	0.791 abc	8.72 bc	0.18 a	25.13 bc
CP 3	0.798 ab	12.16 a	0.16 ab	22.80 c
CP 4	0.764 c	9.08 bc	0.21 a	28.67 b
F-test	**	***	***	***

Different letters within each column indicate significance at Tukey's HSD test ($P \leq 0.05$). ** and ***: significant at $P \leq 0.01$ and 0.001, respectively.

Table 3
Growth variables in *Capsicum* spp. plants as affected by the thermal regime and factors interaction (Exp 1).

Genotype	Stem diameter (mm)			Plant height (cm)			Leaves (n. plant^{-1})			Leaf area (cm^2 plant^{-1})		
	$T_{15/5}$	$T_{25/15}$	Mean	$T_{15/5}$	$T_{25/15}$	Mean	$T_{15/5}$	$T_{25/15}$	Mean	$T_{15/5}$	$T_{25/15}$	Mean
'Capsifort'	5.11	5.80	5.45 c	22.7	39.0	30.8 d	17.7	48.3	33.0 d	338	1220	779 e
CB 5	4.44	7.38	5.91 ab	16.4	72.7	44.5 bc	14.4	116.9	65.7 a	412	3122	1767 a
CB 9	5.21	7.07	6.14 ab	21.9	60.0	40.9 c	16.2	43.6	29.9 d	439	1734	1087 d
CP 2	5.25	7.30	6.28 a	27.6	64.1	45.9 ab	20.2	112.4	66.3 a	452	2594	1523 b
CP 3	4.99	6.80	5.89 b	35.4	62.3	48.9 a	20.6	87.1	53.8 b	438	2154	1296 c
CP 4	4.67	6.32	5.50 c	27.3	61.4	44.4 bc	18.8	73.9	46.3 c	399	2009	1204 cd
Mean	4.94 b	6.78 a		25.2 b	59.9 a		18.0 b	80.4 a		413 b	2139 a	
HSD interaction$_{P=0.05}$	0.93			9.04			15.3			431		

$T_{15/5}$: 15–5 °C for 12 h/day; $T_{25/15}$: 25–15 °C for 12 h/day.
Different letters within each main factor indicate significance at Tukey's HSD test ($P \leq 0.05$).

Table 4
Shoot, root and plant biomass of *Capsicum* spp. plants as affected by thermal regime and factors interaction (Exp 1).

Genotype	Shoot biomass (g DW plant^{-1})			Root biomass (g DW plant^{-1})			Plant biomass (g DW plant^{-1})		
	$T_{15/5}$	$T_{25/15}$	Mean	$T_{15/5}$	$T_{25/15}$	Mean	$T_{15/5}$	$T_{25/15}$	Mean
'Capsifort'	1.63	3.04	2.34 d	0.57	0.49	0.53 d	2.20	3.54	2.87 c
CB 5	1.72	7.73	4.72 b	0.42	1.39	0.91 bc	2.14	9.12	5.63 b
CB 9	3.51	5.05	4.28 c	0.78	0.86	0.82 c	4.29	5.90	5.10 b
CP 2	3.51	7.54	5.53 a	1.29	1.56	1.43 a	4.80	9.10	6.95 a
CP 3	3.60	6.91	5.26 a	1.39	1.57	1.48 a	4.99	8.49	6.74 a
CP 4	3.00	6.52	4.76 b	0.73	1.12	0.93 b	3.74	7.64	5.69 b
Mean	2.83 b	6.13 a		0.86 b	1.16 a		3.69 b	7.30 a	
HSD interaction$_{P=0.05}$	1.09			0.26			1.05		

$T_{15/5}$: 15–5 °C for 12 h/day; $T_{25/15}$: 25–15 °C for 12 h/day.
Different letters within each main factor indicate significance at Tukey's HSD test ($P \leq 0.05$).

Table 5
Physiological, growth and developmental variables in grafted *Capsicum* spp. plants exposed to suboptimal temperature regime (15–5 °C for 12 h/day) (Exp 2).

Graft combination	F_V/F_M (adimensional)	A (μmol CO_2 m^{-2} s^{-2})	gs (mol H_2O m^{-2} s^{-1})	EL (%)	Leaf proline content (μmol g^{-1} FW)	Plant height (cm)	Leaves (n. plant^{-1})	Leaf area (cm^2 plant^{-1})	SLA (cm^2 g^{-1})	Shoot biomass (g DW plant^{-1})	Root biomass (g DW plant^{-1})	Plant biomass (g DW plant^{-1})
'Tiberio'	0.778 b	6.60 c	0.05 b	27.08 a	2.78 a	14.5 d	13.7 c	207 e	219 c	1.47 c	0.51 bc	1.97 d
'Tiberio'/'Capsifort'	0.792 ab	7.55 bc	0.08 ab	23.95 ab	1.06 d	16.1 cd	16.6 ab	411 ac	265 b	2.15 ab	0.70 b	2.85 b
'Tiberio'/CB 5	0.795 ab	6.22 c	0.05 b	27.04 a	1.46 c	18.3 ab	15.7 b	367 c	325 a	1.72 bc	0.45 c	2.17 cd
'Tiberio'/CB 9	0.805 a	9.55 ab	0.06 b	18.76 bc	1.25 cd	19.9 a	17.4 a	424 ab	273 b	2.51 a	0.96 ab	3.47 a
'Tiberio'/CP 2	0.792 ab	8.09 ac	0.06 b	16.82 c	1.29 cd	17.8 bc	15.3 b	262 d	195 c	2.13 ab	0.59 bc	2.72 bc
'Tiberio'/CP 3	0.790 ab	10.69 a	0.12 a	25.64 ab	1.53 c	18.0 b	16.3 ab	463 a	288 ab	2.44 a	1.07 a	3.51 a
'Tiberio'/CP 4	0.797 ab	7.82 ac	0.12 a	26.39 a	2.23 b	17.3 bc	15.7 b	386 bc	305 ab	1.78 bc	0.71 b	2.49 bd
F-test	*	**	**	***	***	**	**	***	***	***	**	***

Different letters within column indicate significance at Tukey's HSD test ($P \leq 0.05$). F_V/F_M: chlorophyll *a* fluorescence; A: net photosynthesis rate; gs: stomatal conductance; EL: electrolyte leakage; SLA: specific leaf area. *, ** and ***: significant at $P \leq 0.05$, 0.01 and 0.001, respectively.

average) (Table 5). Moreover, all the grafting combinations showed a higher leaf number compared to the ungrafted plants, peaking when the scion was grafted onto CB 9, 'Capsifort' and CP 3 (16.8 leaves plant^{-1}, on average), whereas the ungrafted control proved the least values in terms of leaf number and leaf area (13.7 and 207 cm^2 plant^{-1}, respectively) (Table 5). This last variable proved the highest values when the scion was grafted onto CB 9, CP 3 and 'Capsifort' (433 cm^2 plant^{-1}, on average), i.e., a 2.1-fold higher leaf area than 'Tiberio' (Table 5). Specific leaf area was higher in almost all grafted plants compared to the ungrafted ones, with the highest value recorded in 'Tiberio'/CB 5 (325 cm^2 g^{-1}) which was 48.4 and 22.6 % higher than that recorded in 'Tiberio' and 'Tiberio'/'Capsifort', respectively (Table 5). According to the ANOVA, the rootstock significantly influenced the biomass production and partitioning. Indeed, the shoot biomass recorded upon grafting onto CB 9, CP 3, 'Capsifort' and CP 2 was, on average, 57.5 % higher than the ungrafted 'Tiberio'. No differences were observed between control, 'Tiberio'/CB 5 and 'Tiberio'/CP 4 (Table 5). The variations in root biomass were clearly noticeable, particularly in the 'Tiberio'/CP 3 combination, where the values were 110 % and 53 % higher than those recorded when the scion was either ungrafted or grafted onto 'Capsifort', respectively (Table 5). The highest total biomass production was recorded in 'Tiberio'/CB 9 and 'Tiberio'/CP 3 (+77 and +22 %, compared to 'Tiberio' and 'Tiberio'/'Capsifort', respectively) (Table 5).

3.3. Agronomical evaluation of C. annuum 'Tiberio' F_1 grafted onto high altitude accessions under cold Mediterranean greenhouse conditions (Exp 3)

3.3.1. Yield and related components

The highest total and marketable yields were recorded when the scion 'Tiberio' was grafted onto 'Capsifort', CB 5, CB 9, and CP 3, with an

Table 6
Yield and yield components of 'Tiberio' F_1 grafted onto *Capsicum* spp. (Exp 3).

Graft combination	Yield (g plant^{-1})			Fruits (n. plant^{-1})			Fruits FW (g)	
	Total	Marketable	Discarded	Total	Marketable	Discarded	Marketable	Discarded
'Tiberio'	1543 b	1338 b	205 c	8.2 ac	5.5 bc	2.7 b	243 cd	76 de
'Tiberio'/'Tiberio'	1494 b	1355 b	139 d	7.3 bc	5.5 bc	1.8 e	246 cd	77 cd
'Tiberio'/'Capsifort'	2370 a	2064 a	306 a	10.2 a	7.2 a	3.0 a	287 a	102 a
'Tiberio'/CB 5	2218 a	1933 a	285 ab	10.5 a	7.5 a	3.0 a	258 bc	95 ab
'Tiberio'/CB 9	2203 a	1925 a	278 b	9.9 a	6.9 ab	3.0 a	279 ab	93 ab
'Tiberio'/CP 2	1054 c	905 b	149 d	6.3 c	4.0 c	2.3 c	226 d	65 e
'Tiberio'/CP 3	2158 a	2020 a	138 d	9.2 ab	7.1 ab	2.1 cd	285 a	66 de
'Tiberio'/CP 4	1470 b	1256 b	214 c	7.4 bc	5.0 c	2.4 d	251 bd	89 bc
F-test	***	***	***	**	**	***	**	***

Different letters within each column indicate significance at Tukey's HSD test ($P \leq 0.05$). ** and ***: significant at $P \leq 0.01$ and 0.001, respectively.

average of 2237 and 1986 g plant^{-1}, respectively (Table 6). However, the combinations 'Tiberio'/'Capsifort', 'Tiberio'/CB 5 and 'Tiberio'/CB 9 showed the highest discarded yield, ranging from 278 to 306 g plant^{-1}, whereas the self-grafted scion, along with 'Tiberio'/CP 2 and 'Tiberio'/CP 3 showed the least values (142 g plant^{-1}, on average) (Table 6). The yield performances seemed closely related to the number of fruits per plant both in terms of total and marketable fruits. Specifically, those graft combination achieving both the highest total and marketable yield (i.e., when the pepper plants were grafted onto 'Capsifort', CB 5, CB 9 and CP 3,) displayed the highest total and marketable fruits per plant too, with average values equal to 9.9 and 7.2 fruit plant^{-1}, respectively. On the other hand, the graft combinations 'Tiberio'/'Capsifort', 'Tiberio'/CB 5, and 'Tiberio'/CB 9 had the highest number of discarded fruits per plant (3.0, on average) with an average weight of discarded fruits equal to 97 g (Table 6).

3.3.2. *Fruit quality traits*

Regarding fruit quality, there were no significant differences in fruit dry weight and total soluble solids. However, statistical differences were recorded in titratable acidity and DPPH antioxidant capacity (Table 7). Specifically, 'Tiberio'/CP 2 fruits showed lower titratable acidity values than 'Tiberio', whereas 'Tiberio'/CB 9 yielded fruits with the highest DPPH value (545 µmol TE kg^{-1} FW) (Table 7).

4. Discussion

Many vegetable crops originating from tropical and subtropical areas are subject to growth reduction and severe physiological disorders when exposed to chilling temperatures. Consequently, limitation of crop performance results in a lower yield potential (An et al., 2022). The use of chill-adapted rootstocks seems to be a fast and efficient tool for improving the suboptimal temperature tolerance of elite commercial cultivars. However, the *Capsicum* spp. genetic resources useful for this purpose are currently limited and need to be assessed extensively.

Table 7
Main fruit quality traits of 'Tiberio' F_1 grafted onto *Capsicum* spp. (Exp 3).

Graft combination	Dry matter content (%)	Total soluble solids (°Brix)	Titratable acidity (g citric acid equivalent L^{-1})	DPPH (µmol Trolox equivalent kg^{-1} FW)
'Tiberio'	7.1	5.8	1.57 a	473 bc
'Tiberio'/'Tiberio'	7.0	5.4	1.47 ab	408 c
'Tiberio'/'Capsifort'	7.0	5.4	1.31 ab	403 c
'Tiberio'/CB 5	6.8	5.5	1.28 ab	528 ab
'Tiberio'/CB 9	6.7	5.5	1.45 ab	545 a
'Tiberio'/CP 2	6.7	5.1	1.21 b	464 bc
'Tiberio'/CP 3	6.7	5.4	1.34 ab	466 bc
'Tiberio'/CP 4	7.3	5.5	1.32 ab	438 c
F-test	NS	NS	**	***

Different letters within each column indicate significance at Tukey's HSD test ($P \leq 0.05$). ** and ***: significant at $P \leq 0.01$ and 0.001, respectively. NS: not significant.

In experiment 1, we analyzed the growth of high-altitude accessions belonging to *C. baccatum* and *C. pubescens* under two different thermal regimes, in comparison to 'Capsifort' as control, i.e., a widespread commercial pepper rootstock in South Italy. Plant growth, expressed as total biomass, was higher than control in all accessions under optimal thermal conditions, whereas under suboptimal ones only CB 9 CP 2, CP 3 and CP 4 accessions displayed a superior biomass production. Since the reduction in total biomass due to low temperatures was significant in all treatments except for 'Capsifort' and CB 9, a higher total biomass production under low temperature seems, in our experimental conditions, more related to the intrinsic plant vigor rather than to the adaptability to suboptimal temperatures. The higher biomass production was related both to shoot and root biomass. Root growth in CP 2 e CP 3 was not influenced by the colder temperature, so that both accessions displayed the highest root biomass under chilling conditions. This has important implications from a practical viewpoint, since a higher root biomass correlates with increased water and ion absorption from the growth substrates, a feature contributing to mitigate the adverse effects of chilling stress on vegetables (Ahn et al., 1999; Davis et al., 2008). This is an important aspect when the aim is to select new rootstocks, since different authors attribute the improved growth of grafted plants to these greater nutrients availability or absorption rate (Bristow et al., 2021; De Swart et al., 2006; Mauro et al., 2020a).

In this regard, it is also important to consider the plant photosynthetic efficiency, since one of the main negative effects of suboptimal temperatures is represented by the increased production of reactive oxygen species (ROS). Among ROS, hydrogen peroxide and superoxide have a remarkably detrimental effect on PSII. Hence, chlorophyll fluorescence parameters related to Photosystem II efficiency functionality, typically decrease under chilling stress (Kalaji et al., 2017). Our findings show that CB 9 and CP 3 maintained a higher photosynthetic rate than 'Capsifort' under low temperature, a condition which was reflected in their greater plant biomass. This indicates that CB 9 and CP 3 may have a greater capacity to buffer the chilling stress compared to 'Capsifort'. This hypothesis is further supported by the higher chlorophyll *a* fluorescence and lower electrolyte leakage values in CB 9 and CP 3. This aligns with previous studies correlating lower electrolyte leakage values to greater stress tolerance (Gisbert-Mullor et al., 2021; Korkmaz et al., 2010).

Further considerations aimed at evaluating the scion-rootstock affinity and performance are essential, especially when the objective is to increase the tolerance of the plant to complex stresses such as low temperature. As reported by other authors, grafting commercial cultivars onto appropriate rootstocks improves several aspects related to plant development, and consequently results in a greater tolerance of the plant to suboptimal temperatures (Gisbert-Mullor et al., 2021; Venema et al., 2008). Under stress condition, this beneficial influence is generally highlighted by a higher relative growth rate and/or leaf area. These parameters could suggest a lower stress incidence, which can also be measured through various stress indicators like electrolyte leakage. In Exp 2, we observed that the combinations 'Tiberio'/CB 9 and 'Tiberio'/CP 3 had higher total biomass compared to control and commercial rootstock. This effect could potentially be attributed to the greater number of leaves per plant and/or larger leaf area when compared to control. Additionally, our findings suggest that CB 9 and CP 3 may have a greater capacity to mitigate the negative effects of low temperature on 'Tiberio', as evidenced by their higher net photosynthesis. A clear influence of the graft combination was also observed for leaf proline content. The role of proline in higher plants is well described and reported by different authors (Ashraf and Foolad, 2007; Mattioli et al., 2009). Our results indicate that grafting 'Tiberio' onto different rootstocks resulted in decreased leaf proline content, with only 'Tiberio'/CB 9 and 'Tiberio'/CP 2 displaying a proline content similar to the 'Tiberio'/'Capsifort' combination. This seems in contrast to the findings of Penella et al. (2017), who observed higher proline accumulation in grafted pepper under water and salt stress. It is important to note that while proline accumulation is frequently associated with enhanced plant responses to environmental stressors, this correlation is not always applicable. Several studies have demonstrated higher proline accumulation in stress-sensitive plants compared to tolerant ones (Ashraf and Foolad, 2007). Moreover, in our experiment, a set of negative correlations emerged between proline, on one hand, and total DW (-0.553**), plant height (-0.612**), and number of leaves (-0.680***) on the other (data not shown). Based on these correlations, it appears that a higher leaf proline concentration, at least in our experimental conditions, is primarily attributable to a greater susceptibility rather than to an increased tolerance to chilling stress. Additionally, our findings suggest that the scion × rootstock interaction is crucial in determining the accumulation of proline in scion leaves. These results highlight the need to carefully consider both scion and rootstock when evaluating the impact of environmental stressors on plant growth and development.

Numerous studies have demonstrated the significant impact of the scion × rootstock interactions on important crop traits, such as yield and product quality. This critical feature is pivotal in the selection and release of new rootstocks. By carefully assessing the scion × rootstock interaction, we can better understand how different combinations can influence plant growth, development, and ultimately, the productivity and quality of the final product (Mauro et al., 2020c). In the third experiment, we intended to check if the use of the studied accessions as rootstocks can be considered adaptable to greenhouse conditions common in the Mediterranean areas. Indeed, other authors reported a reduction in yield and quality as a consequence of grafting tomato onto a high-altitude *Solanum habrochaites* accession (Ntatsi et al., 2014). In this sense, the data we collected provide information regarding the performance of the high-altitude accessions, particularly with regard to their influence on yield and fruit quality. First of all, no differences were observed between ungrafted and self-grafted 'Tiberio', while the variations observed between the different graft combinations were more noteworthy. Our results show a significant increase in total and marketable yield, compared to ungrafted control, only when 'Tiberio' was grafted onto 'Capsifort', CB 5, CB 9, and CP 3. The increase in marketable yield of pepper grafted onto suitable rootstocks is a well-known finding reported in several studies (Aidoo et al., 2019; Gisbert-Mullor et al., 2020; Leal-Fernández et al., 2013). In line with these researches, the increase in marketable yield we recorded was related both to a higher number of fruits per plant and to a higher average fruit weight. The positive effects of convenient scion-rootstock interactions have been observed also at the photosynthetic level. Liu et al. (2011) reported an increase in net photosynthesis rate, stomatal conductance, concentration of intercellular CO_2 and transpiration rate as a consequence of grafting muskmelon onto two different interspecific rootstocks. Our experiment revealed similar results for 'Tiberio' grafted onto 'Capsifort', CB 5, CB 9, and CP 3, but not when CP 2 and CP 4 were used as rootstocks. It is possible that the performance of these two graft combinations was influenced by a lower adaptability of their respective rootstocks to the experimental climatic conditions. These findings suggest that careful consideration should be given to the selection of rootstocks based on their specific traits, including their adaptability to local environmental conditions. By doing so, we can improve the overall performance of grafted plants and enhance their productivity under challenging conditions.

Regarding the main fruit qualitative traits, slight differences were recorded for titratable acidity and antioxidant activity. Only 'Tiberio'/CB 9 displayed a higher antioxidant activity than ungrafted control, while taking into account the commercial rootstock, both *C. baccatum* accessions determined a higher fruit antioxidant activity than 'Capsifort'. Hence, in line with our prior experimental results, this suggests a highly positive scion × rootstock interaction. However, considering other variables such as fruit dry matter content or total soluble solids, the influence of grafting combination was not significant. Conflicting findings are reported in literature. For instance, results showing that grafting determines a higher dry matter content of the fruit were reported by Sánchez-Torres et al. (2016) in pepper cultivars 'Almuden' and 'Coyote' grafted onto 'Foc' and 'Charlot'. Differently Colla et al. (2008) found no effects of grafting on fruit dry weight, total soluble solids and titratable acidity of two pepper cultivars ('Edo' and 'Lux') grafted onto five commercial rootstocks. These data indicate a significant impact of the scion × rootstock interaction on pepper fruit quality, which necessitates to be assessed case-by-case.

5. Conclusions

Our study revealed that the *Capsicum* accessions tested in our experiments exhibited different levels of adaptability to low temperature conditions. These findings have significant implications for breeding programs focused on enhancing low temperature tolerance in pepper crop through grafting. Among the accessions tested CB 9 and CP 3 consistently displayed positive performance in terms of plant growth (Exp 1 and 2) or yield (Exp 3), indicating their potential as rootstocks for improving plant productivity even under normal field conditions and mitigating the risk of yield loss due to low temperatures. While CP 2 demonstrated promising results under low temperature conditions, this genotype produced less than the commercial rootstock under the greenhouse conditions. On the other hand, CB 5 showed the opposite behavior. These results underscore the importance of scion × rootstock interaction as well as the scion × rootstock × environment interaction. They highlight the need to validate the usefulness of new rootstocks in several stages and under different conditions to determine their practical applicability and potential benefits.

CRediT authorship contribution statement

Michele Agnello: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Rosario Paolo Mauro:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Claudio Cannata:** Writing – review & editing, Writing – original draft, Data curation. **Ivana Puglisi:** Formal analysis, Data curation. **Francesco Giuffrida:** Writing – review & editing, Supervision, Methodology, Investigation, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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