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Roots play a key role in drought-tolerance of poplars as suggested by reciprocal grafting between male and female clones

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Abstract

Drought stress influences the growth of plants and thus grafting has been widely used to improve tolerance to abiotic stresses. Poplars possess sex-specific responses to drought stress, but how male or female rootstock affect the grafted plant is little known. To explore the mechanisms underlying changes in drought tolerance caused by grafting, we investigated the changes in growth, leaf traits, gas exchange and antioxidant enzyme activities of reciprocally grafted seedlings between *Populus euramericana* cv. "Nanlin895" (NL-895) (female) and *Populus deltiodes* cv."3412" (NL-3412) (male) under water deficit stress with 30% field capacity for 30 d. Results showed that drought stress affected adversely growth, morphological, and physiological characteristics in all seedlings studied. Grafted seedlings with male roots can effectively alleviated the inhibition of growth induced by drought stress, as shown by higher WUE, activities of SOD, POD and CAT, and lower levels of lipid peroxidation. Male seedlings with female roots were found to be less tolerance to drought than non-grafted male clones and female scions with male roots, but more tolerance than non-grafted female clones. This results suggested that drought tolerance of grafted seedlings is primarily caused by the rootstock, although the scion also affects the grafted plant. Thus, paying attention on the root genotype can provide an important means of improving the drought tolerance of poplars.

1. Introduction

Drought is one of the major environmental stresses that limit plant growth and development. During recent years, increasingly frequent drought events in many areas have decreased plant production, affected functional processes, inhibited photosynthesis and led to the accumulation of reactive oxygen species (ROS), even causing plant death (Wang et al., 2017). Strategies that enhance drought resistance differ depending on the intensity and duration of the stress as well as on the species (Yang et al., 2014; Wang et al., 2017). Plants' responses to drought stress are typically morphological, physiological or biochemical. For instance, drought stress can induce decreased height and leaf growth (Jaleel et al., 2009), result in a reduction in the chlorophyll content, photosynthetic and transpiration rates, or cause stomatal closure and limitation of photosynthesis (Wang et al., 2017). However, the damage induced by drought stress can be alleviated by inherent regulation mechanisms, i.e., the activities of the antioxidant defense system, which help the plants to maintain a certain level of physiological activity (Fang and Xiong, 2015).

Grafting is regarded as a rapid tool that can improve abiotic stress tolerance of plants (Flores et al., 2010), which has been used in many fruits and crops, such as tomato, cucumber, apricot, citrus, etc. (Flores et al., 2010; Liu et al., 2016; He et al., 2018; Reig et al., 2018). However, it is very difficult to determine whether grafted plants' responses to environmental stresses are controlled by roots or shoots. Liu et al. (2014) have demonstrated that grafting with rootstock genotypes tolerant to drought could improve drought stress tolerance in tobacco by regulating antioxidant enzyme activities and stress-responsive gene expression. Recent studies have showed that suitable rootstocks could improve resistance to water stress in tomato (Flores et al., 2010). Most previous studies on grafted plants have showed that there is chemical signaling from the root to the shoot, which plays an important role in regulating a plant's morphology and physiology (Orsini et al., 2013; Tsutsui and Notaguchi, 2017). In this regard, grafting a stress-sensitive genotype onto a rootstock with a better stress tolerance could be a useful approach to improve water use efficiency (WUE) and plant productivity (Liu et al., 2016), however, this is well known in horticulture but less in forest trees.

Poplars are important dioecious, and multipurpose woody plants. Their drought tolerance varies greatly among species (Melnikova et al., 2017) and most Populus genotypes with high productivity were drought-susceptible (Monclus et al., 2006). Many studies have showed that male and female poplars have different responses to stress (Xu et al., 2008; Azizi et al., 2017; Melnikova et al., 2017; Chen et al., 2018). Generally, female plants tend to be more responsive and suffer from greater negative effects than do males under unfavorable conditions, while females show better adaptability under favorable conditions (He et al., 2016; Wang et al., 2017; Chen et al., 2018; Yu et al., 2018). Previous study has suggested that male genotype, *Populus deltiodes*, had a strong drought tolerance, which could provide a useful rootstock for enhancing drought tolerance (Han et al., 2019). However, the effect of male genotype as rootstock or scion on the drought resistance of grafted plants should be further discussed. In order to fully interpret morphological and physiological response in responses to water deficit, it is important to understand the roles of both rootstock and scion.

In this study, an excellent female cultivar, *Populus euramericana* cv. "Nanlin895" (NL-895), and an excellent male cultivar, *Populus deltiodes* cv."3412" (NL-3412), were investigated. As the fast-growing tree species with fine timber, they are widely planted in the middle and lower reaches of the Yangtze River, China, and used for the rehabilitation of marginal areas owing to their high survival rate. We reciprocally grafted rootstocks and shoot scions from NL-895 and NL-3412, and aimed to: (1) compare the responses of reciprocally grafted male and female poplars to water deficit stress through an investigation of morphological and physiological characteristics, and (2) to determine whether a rootstock from a tolerant genotype could improve the drought tolerance in the grafted plant.

2. Materials and methods

2.1. Plant material and grafting

Cuttings of female NL-895 and male NL-3412 clones were collected from the Nanjing Forestry University. Cuttings (about 15 cm) from both clones were used as scions and rootstocks in reciprocal grafting: female shoot scion and male rootstock (F/M), and male shoot scion and female rootstock (M/F). Then, non-grafted male (M) and female (F) cuttings, and two types of grafted cuttings were planted in the field in March 2016. The splice grafting technique was used (details in Fig. S1). After sprouting and growing for 30 days, healthy seedlings of approximately equal heights (about 20 cm) were transplanted into plastic pots with 40 cm (height) × 30 cm (diameter). Each pot was filled with 9 kg homogenized mixture of reddish-brown forest soil.

2.2. Drought stress treatments

After 75 days of normal management, 30 healthy plants of each clone or graft combination were divided into two groups with 15 plants in each group, and used for the drought stress and control treatments, respectively. The experiment was initiated on July 15, 2016 and it lasted 60 days. Two watering treatments were supplied: 1) normal watering treatment (CK): field water content equaling 80%; 2) severe drought stress (TR): field capacity of 30%. In the control, the pots were watered to 80% field capacity (soil water content maintained at 31%) every day. In the drought treatment, the pots were watered only to 30% field capacity (soil water content maintained at 14%) every day. The average soil water content was based on measurements of the soil moisture content, which was detected three times per day by a soil moisture probe. Evaporation from the soil surface was prevented by enclosing the pots with plastic bags sealed at the base of the stem of each plant. The pot locations were often randomly changed to reduce the potential effect of microclimate during the experiment. All pots were placed under rain shelters at the Botanical Garden of Zhejiang Agriculture and Forestry University (119°44′ E, 30°16′ N), Hangzhou, China. During the experimental period, the temperature range was 27–33 °C and the relative humidity range was 45–65%.

2.3. Determination of growth parameters and biomass measurements

The growth characteristics, including leaf length and width, plant height and basal diameter, were measured by calipers and a tape measure at 15 d and 30 d during the experiment. From the 3rd fully expanded leaves (counted from the top of the plant), at least 3 mature leaves in low layer per plant were measured. The numbers of leaves per plant were counted. The leaf area was determined by a portable laser area meter (CI-203, CID Inc., USA). The part 1 cm above the graft union was regarded as the “stem” in the grafted plants and the part 2 cm above soil surface was regarded as the “stem” in non-grafted plants (Peng et al., 2013). During the stress experiment, some seedlings withered and even died. At the stressed 15 d, at least 10 seedlings in each group were chosen while only 5 seedlings with at least 5 mature leaves were selected at stressed 30 d. At the end of the experiment, three chosen seedlings were harvested and partitioned into leaves, stems and roots. All samples were dried separately at 80 °C for 48 h to constant weight and weighed. Dry mass accumulation was then calculated.

2.4. Determination of chlorophyll content, gas exchange and WUE

The chlorophyll content and gas exchange were measured at days 15 and 30. Using a portable chlorophyll content instrument (SPAD-502, Japanese), the relative chlorophyll content was measured from 09:00–11:30 a.m. using 10 measurements for each leaf at upper, middle and lower parts of the plants. Three seedlings of each clone and grafted combinations were selected from each treatment. Using a portable photosynthesis system, Li-6400 (Li-Cor Inc. Lincoln, Nebraska, USA), gas exchange parameters were measured at the same time. The leaf temperature was controlled at 28–30 °C with a relative humidity of 36–55% inside the leaf chamber during measurements. The photosynthetic photon flux density was 1200 μmol･m−2･s−1, and the ambient CO2 concentration was 400 ± 5 μmol ･mol−1. The measurements conducted for mature leaves included net photosynthetic rate (Pn), transpiration rate (Tr), stomatal conductance (Gs) and intercellular CO2 concentration (Ci). WUE was calculated according to the following formula: WUE = Pn/Tr.

2.5. Determination of antioxidant enzyme activity

Antioxidant enzyme activities were measured at days 15 and 30 during the experiment. Fresh leaves from the 4th and 5th fully expanded leaves (counted from the top of the plant) of three plants from each treatment were collected and weighed to 1g, then ground in liquid nitrogen, followed by extracting with 50 mM potassium phosphate buffer (pH 7.8) containing 0.1 mM EDTA, 1% (w/v) PVP, 0.1 mM PMSF, and 0.2% (v/v) Triton X100 for the measurements of SOD, POD and CAT. The extractions were centrifuged at 14,000×g for 15 min at 4 °C. The supernatants were collected for the enzyme activity assays. The means ± SE were calculated from the data of at least 3 independent measurements.

The SOD activity was measured following the method described by Beauchamp and Fridovich (1971). One unit of SOD was defined as the enzyme concentration required to inhibit the chromogen produced (NBT) by 50% in 1 min at 560 nm. The POD activity was measured according to the method proposed by Upadhyaya et al. (1985). One unit of POD corresponds to the amount of enzyme producing 1 μmol･min−1 of oxidized guaiacol at 470 nm. The CAT activity was measured following the procedure of Aebi (1984), using UV spectrophotometry by a direct measurement of H2O2 decomposition at 240 nm.

2.6. Determination of lipid peroxidation and relative electric leakage measurements

Lipid peroxidation and relative electrolyte leakage were measured at days 15 and 30. The content of MDA was determined by thiobarbituric acid (TBA) method, as described by Heath and Packer (1968) with a little modification as follows: 0.5 g fresh leaves with 2 ml of 0.1% trichloroacetic acid (TCA) were centrifuged at 14,000 rpm for 15 min. Then, 2 ml of centrifuged supernatant (control plus 2 ml distilled water) was mixed with 2.5 ml 0.5% TBA in 20% TCA and incubated at 95 °C for 15 min, quickly cooled on ice, and then centrifuged at 10,000 rpm for 20 min. Absorbances of the supernatant at 532, 600 and 450 nm were determined. The concentration of MDA was calculated using the following formula: $C\ (\mu mol \cdot l^{-1}) = 6.45(A532\text{-}A600) - 0.56A450$.

The relative electric conductivity (REL) was determined using the conductivity instrument. Briefly, 1g of fresh leaf tissue was immersed in 20 ml deionized water, and then degassed for 15 min. The initial conductivity (E1) of was measured with a conductivity instrument (LC116, Mettler Toledo Instruments Co., Ltd, Shanghai, China) after 30 min. The solution was incubated at 100 °C for 15 min and cooled to room temperature, and the final conductivity (E2) was determined. The conductivity of deionized water was also determined as blank conductivity (E0). The relative conductivity was calculated according to the following formula: relative conductivity (%) = (E1 - E0)/(E2 - E0) × 100%.

2.7. Statistical analysis

Data was analyzed using the statistical software package SPSS 13.0 (SPSS, Chicago, IL, USA). One-way ANOVAs were employed to determine the effects of grafting types (genotypes) or water treatment at each time point. Two-way ANOVAs were used to assess the interaction effects between the grafting type (genotypes) and water treatment. The Duncan test was employed to detect possible differences between the drought stress and control treatments, and grafting combinations (genotypes). Mean values and standard errors were determined for each variable. A significant difference was considered at the $P < 0.05$ level.

3. Results

3.1. Differences in growth traits among graft combinations and non-grafted seedlings

We found interaction effects between genotype and water stress on plant growth traits (except for the basal diameter). Drought decreased leaf length (Fig. 1A and B), leaf width (Fig. 1C and D), leaf area (Fig. 1E and F), the number of leaves (Fig. 1G and H), plant height (Fig. 2A and B) and biomass (Fig. 3) in both non-grafted seedlings and graft combinations. In the well-watered treatment, reciprocally grafted seedlings (M/F and F/M) had significantly lower leaf area at 15 d ($P < 0.05$). By day 30, there were significant differences in most traits between the drought stress treated and control seedlings ($P < 0.05$). Reciprocally grafted seedlings (F/M and M/F) consistently showed greater decreases in growth parameters than the control plants when compared with non-grafted males and females under water deficit conditions. At the end of the experiment, the total biomass of F, M, M/F and F/M was significantly decreased by 75.33%, 48.06%, 72.81% and 55.54% when compared to control, respectively ($P < 0.05$) (Fig. 3).

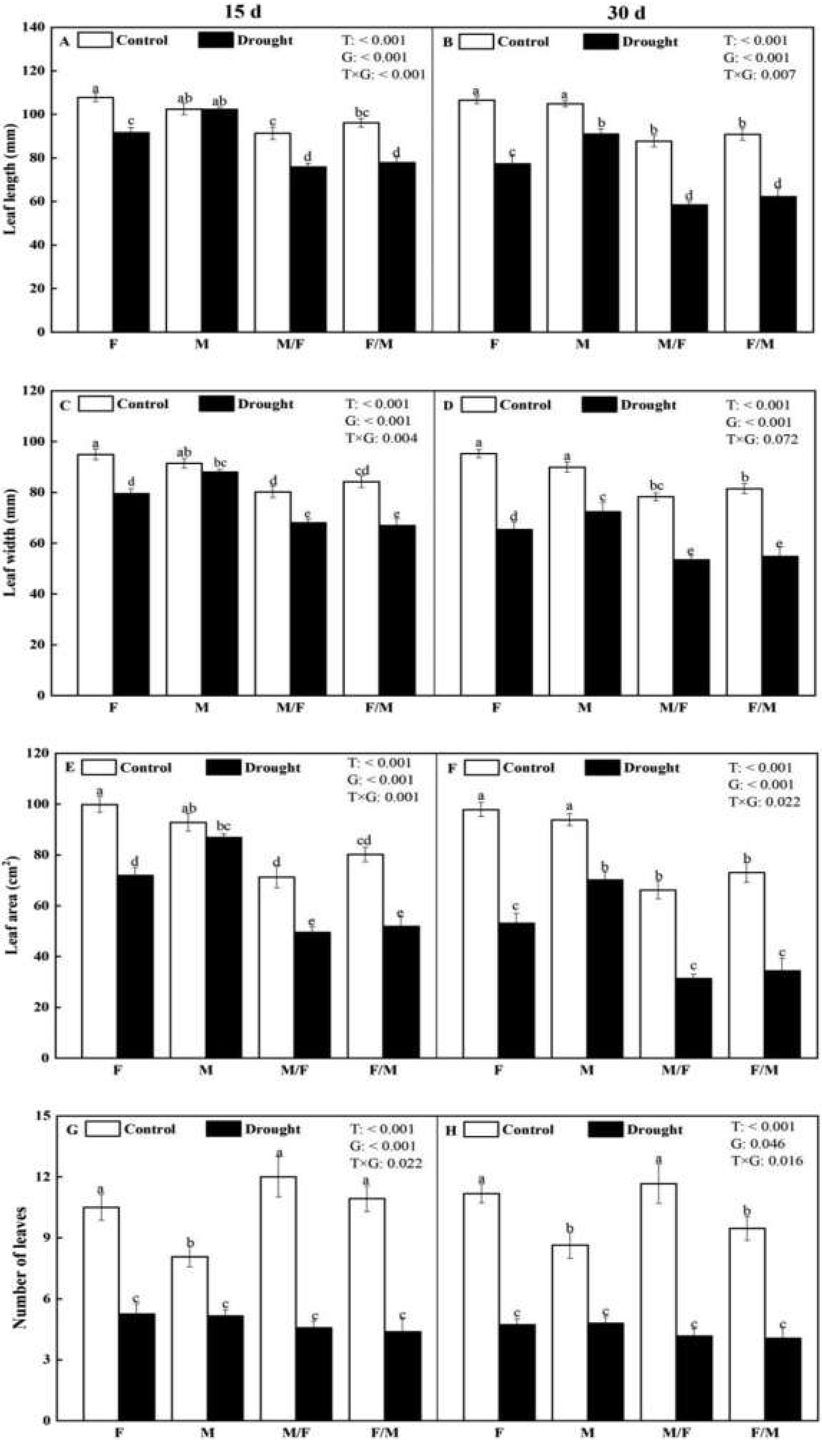
15 d
30 d
A
B
C
D
E
F
G
H
Control
Drought
T: < 0.001
G: < 0.001
T×G: < 0.001
T: < 0.001
G: < 0.001
T×G: 0.007
T: < 0.001
G: < 0.001
T×G: 0.004
T: < 0.001
G: < 0.001
T×G: 0.072
T: < 0.001
G: < 0.001
T×G: 0.001
T: < 0.001
G: < 0.001
T×G: 0.022
T: < 0.001
G: < 0.001
T×G: 0.022
T: < 0.001
G: 0.046
T×G: 0.016
Leaf length (mm)
Leaf width (mm)
Leaf area (cm^2)
Number of leaves
0
20
40
60
80
100
120
140
3
6
9
12
15
F
M
M/F
F/M
a
ab
bc
c
cd
d
e
b

Fig. 1. Leaf length (A, B), leaf width (C, D), leaf area (E, F) and number of leaves (G, H) in female genotype, *Populus euramericana* cv. "Nanlin895" (NL-895) (F), male genotype *Populus deltiodes* cv.*"3412"* (NL-3412) (M), and reciprocal grafted seedlings between male and female clones under water deficit stress. The values are shown as mean ± SE (15 d, $n \geq 10$; 30 d, $n = 5$). Different letters indicate significant differences among treatments according to Duncan's test at a significance level of $p < 0.05$. M/F, male shoot scion and female rootstock; F/M, female shoot scion and male rootstock; Control, normal watering treatment (field water content equaling 80%); Drought, severe drought stress (field capacity of 30%); T, treatment (drought) effect; G, genotype effect; T × G, treatment (drought) × genotype effect.

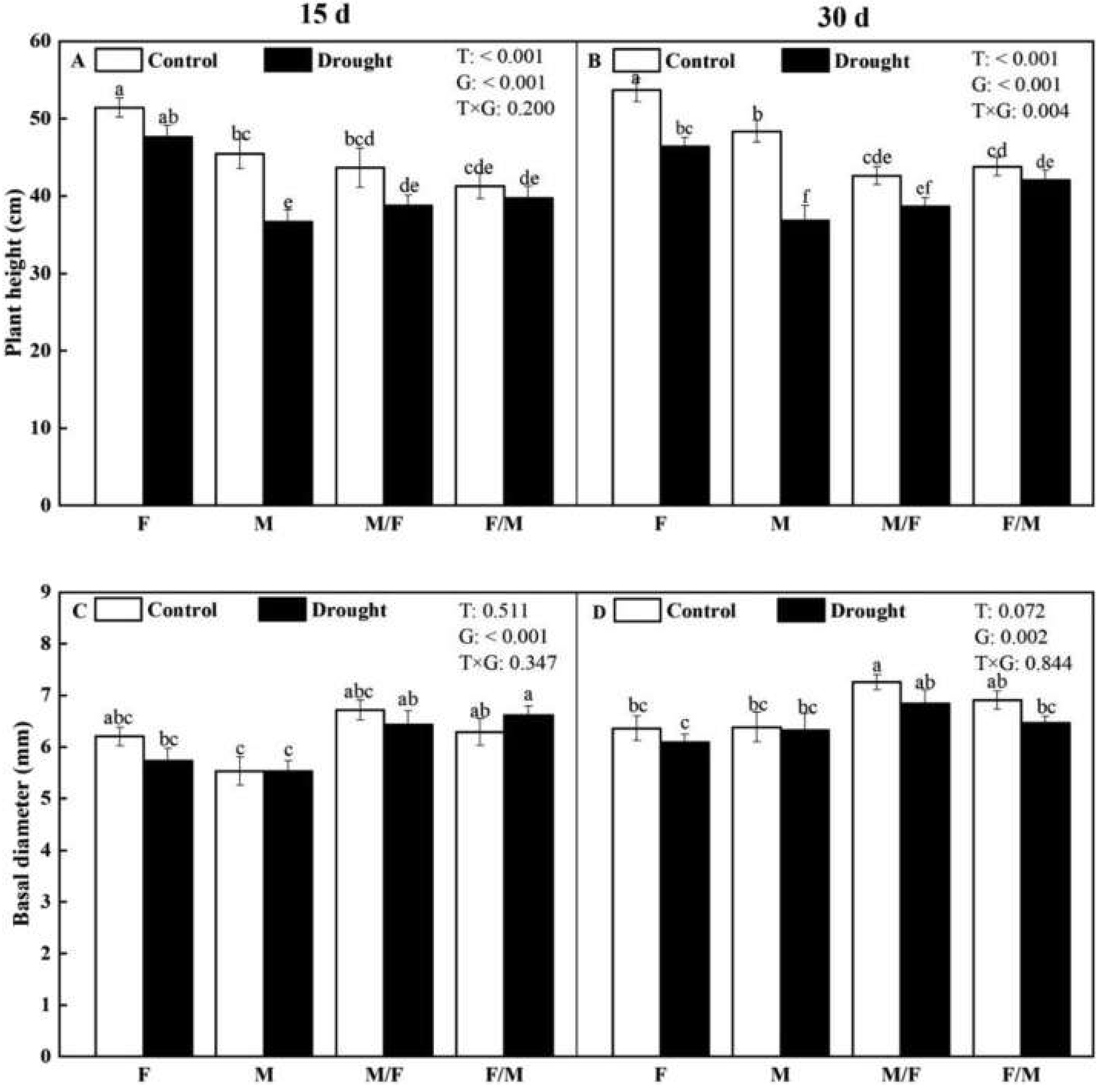

Fig. 2. Plant height (A, B) and basal diameter (C, D) in female genotype, *Populus euramericana* cv. "Nanlin895" (NL-895) (F), male genotype *Populus deltiodes* cv.*"3412"* (NL-3412) (M), and reciprocal grafted seedlings between male and female clones under water deficit stress. The values are shown as mean ± SE (15 d, $n \geq 10$; 30 d, $n = 5$). Different

letters indicate significant differences among treatments according to Duncan's test at a significance level of $p < 0.05$. M/F, male shoot scion and female rootstock; F/M, female shoot scion and male rootstock; Control, normal watering treatment (field water content equaling 80%); Drought, severe drought stress (field capacity of 30%); T, treatment (drought) effect; G, genotype effect; T × G, treatment (drought) × genotype effect.

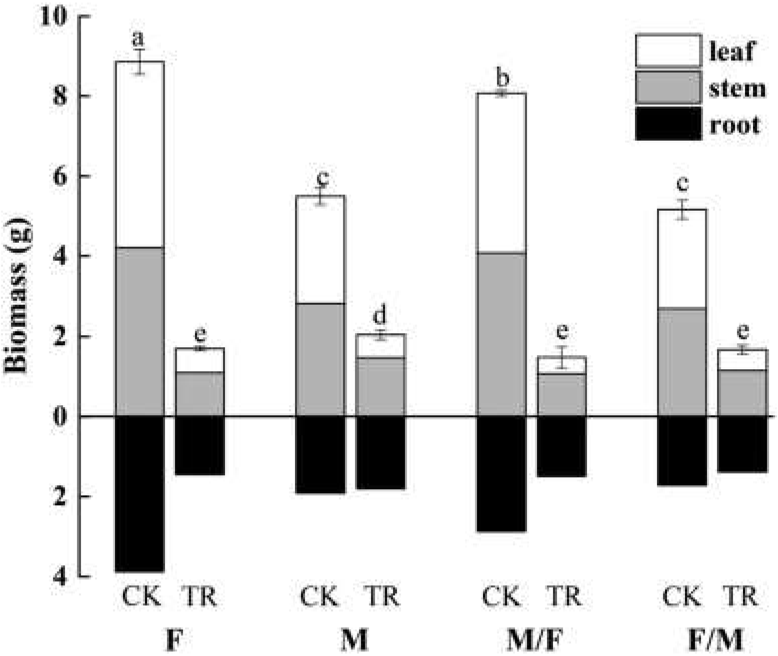

Fig. 3. Biomass in female genotype, *Populus euramericana* cv. "Nanlin895" (NL-895) (F), male genotype *Populus deltiodes* cv.*"*3412*"* (NL-3412) (M), and reciprocal grafted seedlings between male and female clones under water deficit stress. The values are shown as mean ± SE ($n = 3$). Different letters indicate significant differences among treatments according to Duncan's test at a significance level of $p < 0.05$. M/F, male shoot scion and female rootstock; F/M, female shoot scion and male rootstock; Control, normal watering treatment (field water content equaling 80%); Drought, severe drought stress (field capacity of 30%).

3.2. Chlorophyll content, gas exchange and WUE under drought stress

Water deficit had no significant effect on the relative chlorophyll content of seedlings compared to the control plants (Fig. 4A and B). There are no significant differences in P_n values between grafted and non-grafted seedlings under water deficit treatment (Fig. 5A and B), but P_n of stressed seedlings significantly decreased when compared to the control plants (Fig. 5B), with decreases by 24.91% (M/F) and 21.46% (F/M), and by 35.76% in females and 19.49% in males at 30 d. A decrease in T_r was observed in both grafted and non-grafted seedlings at 30 d stress ($P < 0.05$) (Fig. 5D). T_r value of F/M decreased more than that in non-grafted females at 15 d stress, while M/F decreased less than that in non-grafted males at 30 d stress. By day 30, the T_r value of grafted seedlings was significantly higher than that of males ($P < 0.05$) and the T_r value of F/M was significant lower than that of M/F ($P < 0.05$) (Fig. 5D). Under drought stress, G_s decreased in female seedlings compared to the control plants (Fig. 5E and F). Whether or not water deficit, there were no significant differences in G_s between non-grafted and grafted seedlings ($P > 0.05$) at 15 d (Fig. 5E). The G_s value of M/F was significantly lower than that of male seedling at 30 d ($P < 0.05$) (Fig. 5F). Compared to non-grafted seedlings, C_i of M/F showed a significant decrease under stress conditions at 15 d ($P < 0.05$) (Fig. 5G). By day 30, the C_i values of graft combinations and non-grafted seedlings showed significant differences under drought stress ($P < 0.05$) (Fig. 5H). WUE increased in all seedlings (except M/F) with prolonged drought stress, and seedlings with male root under drought stress had higher increase compared to the control plants (Fig. 4D). WUE of drought-stressed leaves in M/F was much lower than that of stressed males.

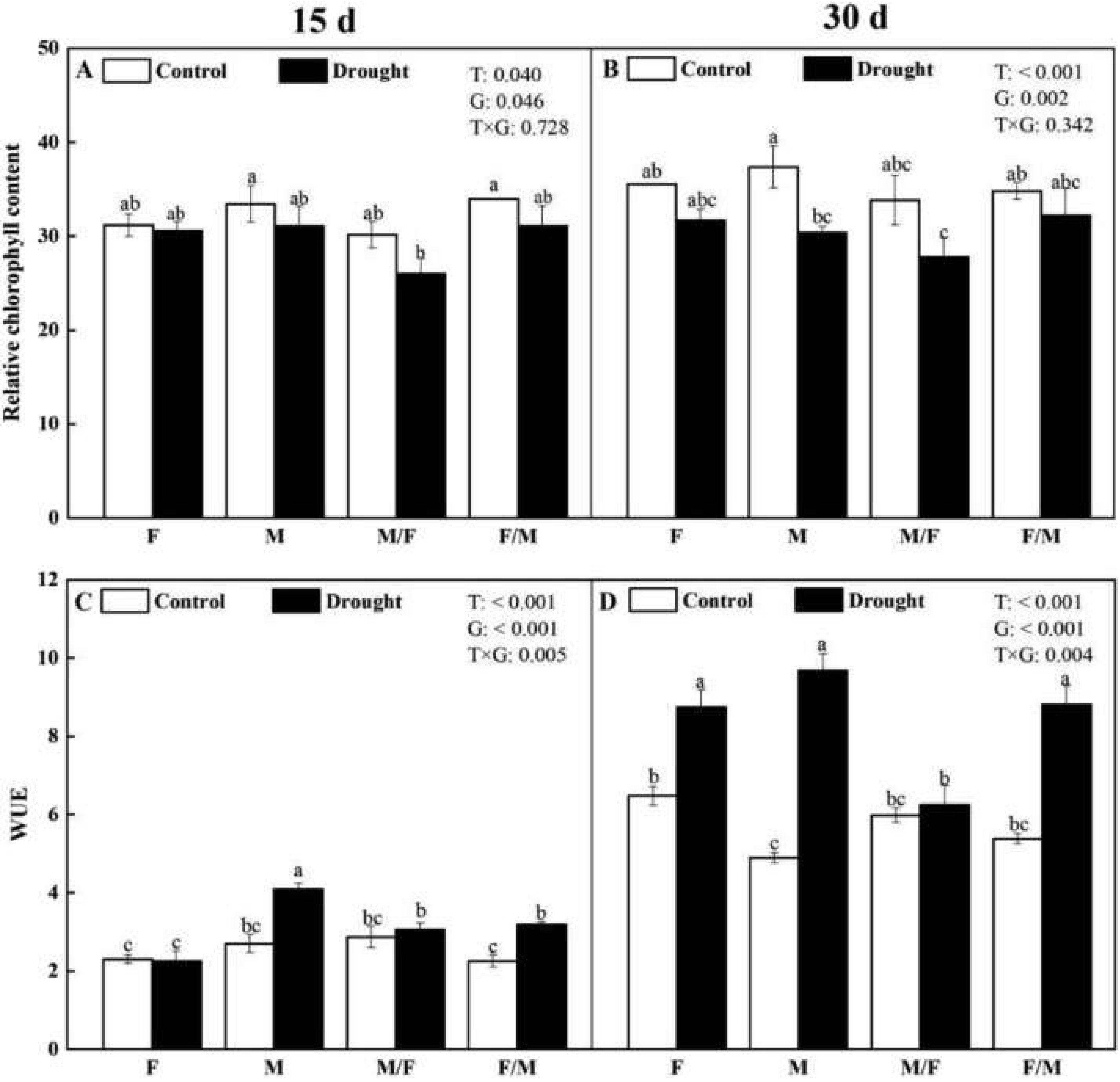

Fig. 4. Relative chlorophyll content (A, B) and WUE (C, D) in female genotype, *Populus euramericana* cv. "Nanlin895" (NL-895) (F), male genotype *Populus deltiodes* cv.*"3412"* (NL-3412) (M), and reciprocal grafted seedlings between male and female clones under water deficit stress. The values are shown as mean ± SE ($n = 3$). Different letters indicate significant differences among treatments according to Duncan's test at a significance level of $p < 0.05$. M/F, male shoot scion and female rootstock; F/M, female shoot scion and male rootstock; Control, normal watering treatment (field water content equaling 80%); Drought, severe drought stress (field capacity of 30%); T, treatment (drought) effect; G, genotype effect; T × G, treatment (drought) × genotype effect.

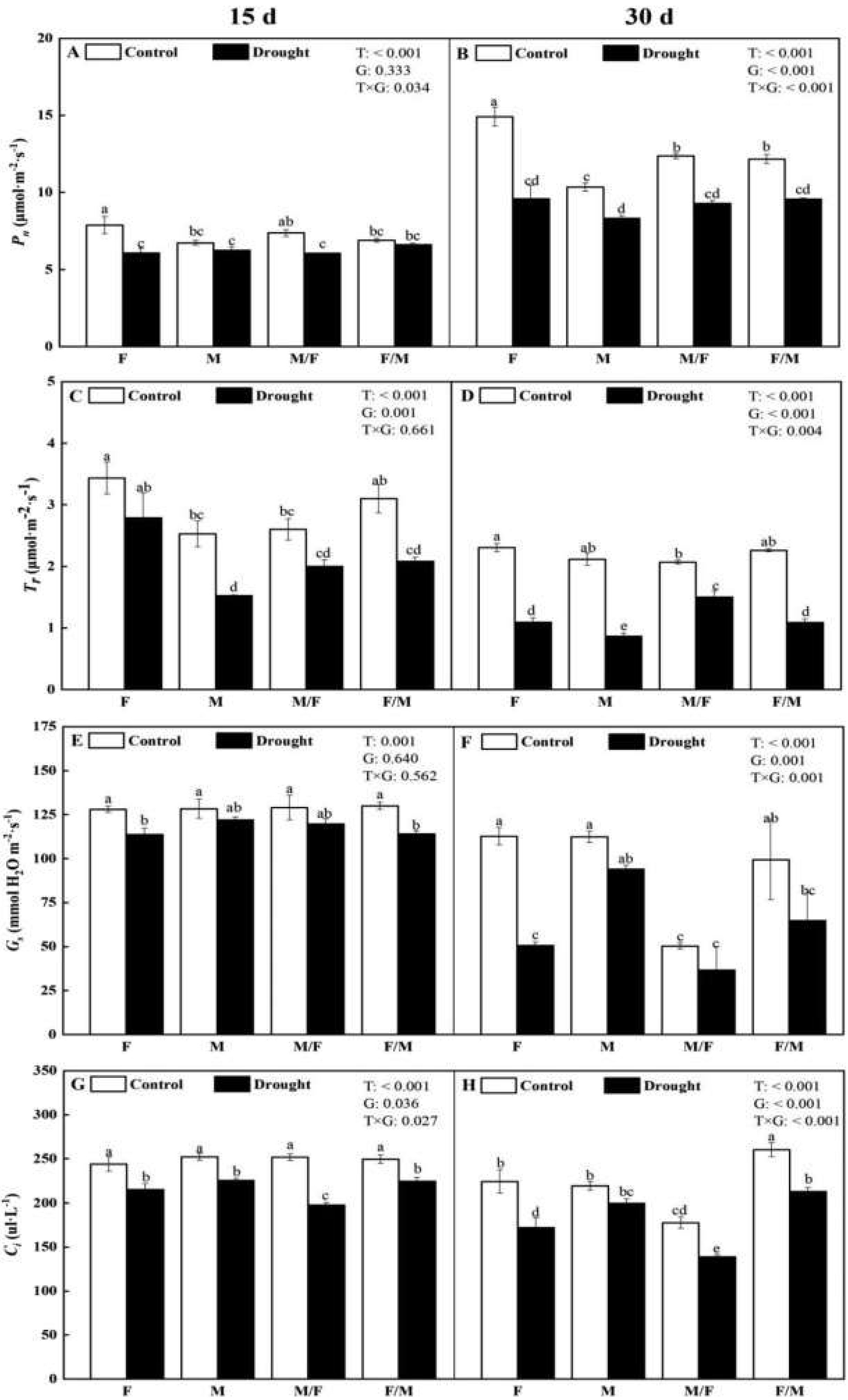
15 d
30 d
A
Control
Drought
T: < 0.001
G: 0.333
T×G: 0.034
B
T: < 0.001
G: < 0.001
T×G: < 0.001
P_n (μmol·m^{-2}·s^{-1})
C
T: < 0.001
G: 0.001
T×G: 0.661
D
T: < 0.001
G: < 0.001
T×G: 0.004
T_r (μmol·m^{-2}·s^{-1})
E
T: 0.001
G: 0.640
T×G: 0.562
F
T: < 0.001
G: 0.001
T×G: 0.001
G_s (mmol H_2O m^{-2}·s^{-1})
G
T: < 0.001
G: 0.036
T×G: 0.027
H
T: < 0.001
G: < 0.001
T×G: < 0.001
C_i (ul·L^{-1})
F
M
M/F
F/M

Fig. 5. Net photosynthetic rate (P_n) (A, B), transpiration rate (T_r) (C, D), stomatal conductance (G_s) (E, F) and intercellular CO_2 concentration (C_i) (G, H) in female genotype, *Populus euramericana* cv. "Nanlin895" (NL-895) (F), male genotype *Populus deltiodes* cv.*"*3412*"* (NL-3412) (M), and reciprocal grafted seedlings between male and female clones under water deficit stress. The values are shown as mean ± SE (*n* = 3). Different letters indicate significant differences among treatments according to Duncan's test at a significance level of $p < 0.05$. M/F, male shoot scion and female rootstock; F/M, female shoot scion and male rootstock; Control, normal watering treatment (field water content equaling 80%); Drought, severe drought stress (field capacity of 30%); T, treatment (drought) effect; G, genotype effect; T × G, treatment (drought) × genotype effect.

3.3. SOD, POD and CAT activities under drought stress

We detected interaction effects on antioxidant enzyme activities among the grafting type, water stress and stress time. Drought stress significantly increased the activities of SOD, POD and CAT ($P < 0.05$) (Fig. 6). Higher values of SOD, POD and CAT were detected in males than in females under drought stress. Compared to the control plants, the activities of SOD, POD and CAT in the grafted F/M plants increased more than those of M/F under water deficit treatment. When drought stress lasted 15 d, the SOD values of M/F and F/M increased by 14.03% and 20.11%, respectively, compared to control treatment (Fig. 6A). The POD activities of M/F and F/M were 25.16% and 20.76% higher under drought stress than in control conditions, respectively ($P < 0.05$) (Fig. 6C). The CAT activities of F/M and M/F seedlings were 42.90% and 31.21% higher under drought stress than in control conditions at 15 d, respectively ($P < 0.05$) (Fig. 6E). The activities of SOD, POD and CAT in all seedlings significantly increased when drought stress lasted to 30 d ($P < 0.05$) (Fig. 6B, D, F). Under drought stress conditions, F/M plants had higher SOD, POD and CAT activities than non-grafted females, while M/F plants had lower antioxidant enzyme activities than non-grafted males (Fig. 6).

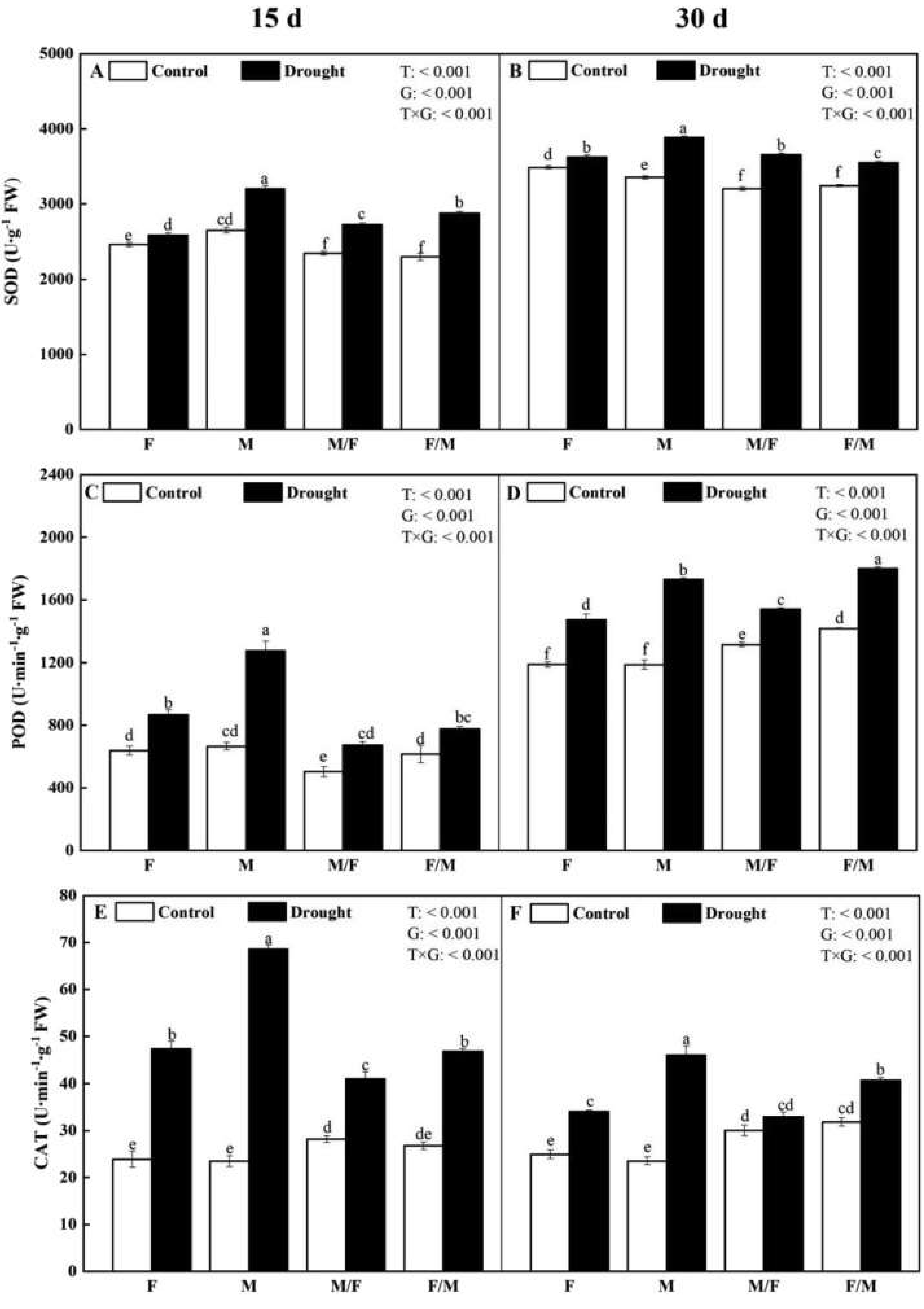
15 d
30 d
A
B
C
D
E
F
Control
Drought
T: < 0.001
G: < 0.001
T×G: < 0.001
SOD (U·g-1 FW)
POD (U·min-1·g-1 FW)
CAT (U·min-1·g-1 FW)
5000
4000
3000
2000
1000
0
2400
2000
1600
1200
800
400
0
80
70
60
50
40
30
20
10
0
F
M
M/F
F/M
e d cd a f c f b
d b e a f b f c
d b cd a e cd d bc
f d f b e c d a
e b e a d c de b
e c e a d cd cd b

Fig. 6. SOD (A, B), POD (C, D) and CAT (E, F) in female genotype, *Populus euramericana* cv. "Nanlin895" (NL-895) (F), male genotype *Populus deltiodes* cv.*"*3412*"* (NL-3412) (M), and reciprocal grafted seedlings between male and female clones under water deficit stress. The values are shown as mean ± SE ($n = 3$). Different letters indicate significant differences among treatments according to Duncan's test at a significance level of $p < 0.05$. M/F, male shoot scion and female rootstock; F/M, female shoot scion and male rootstock; Control, normal watering treatment (field water content equaling 80%); Drought, severe drought stress (field capacity of 30%); T, treatment (drought) effect; G, genotype effect; T × G, treatment (drought) × genotype effect.

3.4. MDA contents and relative electric conductivity

The MDA content of all drought-stressed leaves increased at 15 d and 30 d (Fig. 7A and B). At 15 d, the MDA contents of the drought-stressed female, male, M/F and F/M seedling leaves were 42.84%, 25.41%, 24.07% and 16.50% higher than those of control plants, respectively ($P < 0.05$) (Fig. 7A). The same trend was visible at 30 d (Fig. 7B). Compared to the controls, the MDA content of M/F increased more than that of F/M under stress. The MDA value of F/M and M/F was higher than that of males and lower than that of females under water deficit. Compared with the control plants, REL increased with prolonged drought stress, but the increase was lower in males than in females (Fig. 7C and D). The REL value of both F/M and M/F was higher than that of males and lower than that of females under water deficit at 15 d (Fig. 7C). By 30 d of stress, the REL value of both F/M and M/F was higher than that of females and males under drought stress (Fig. 7D).

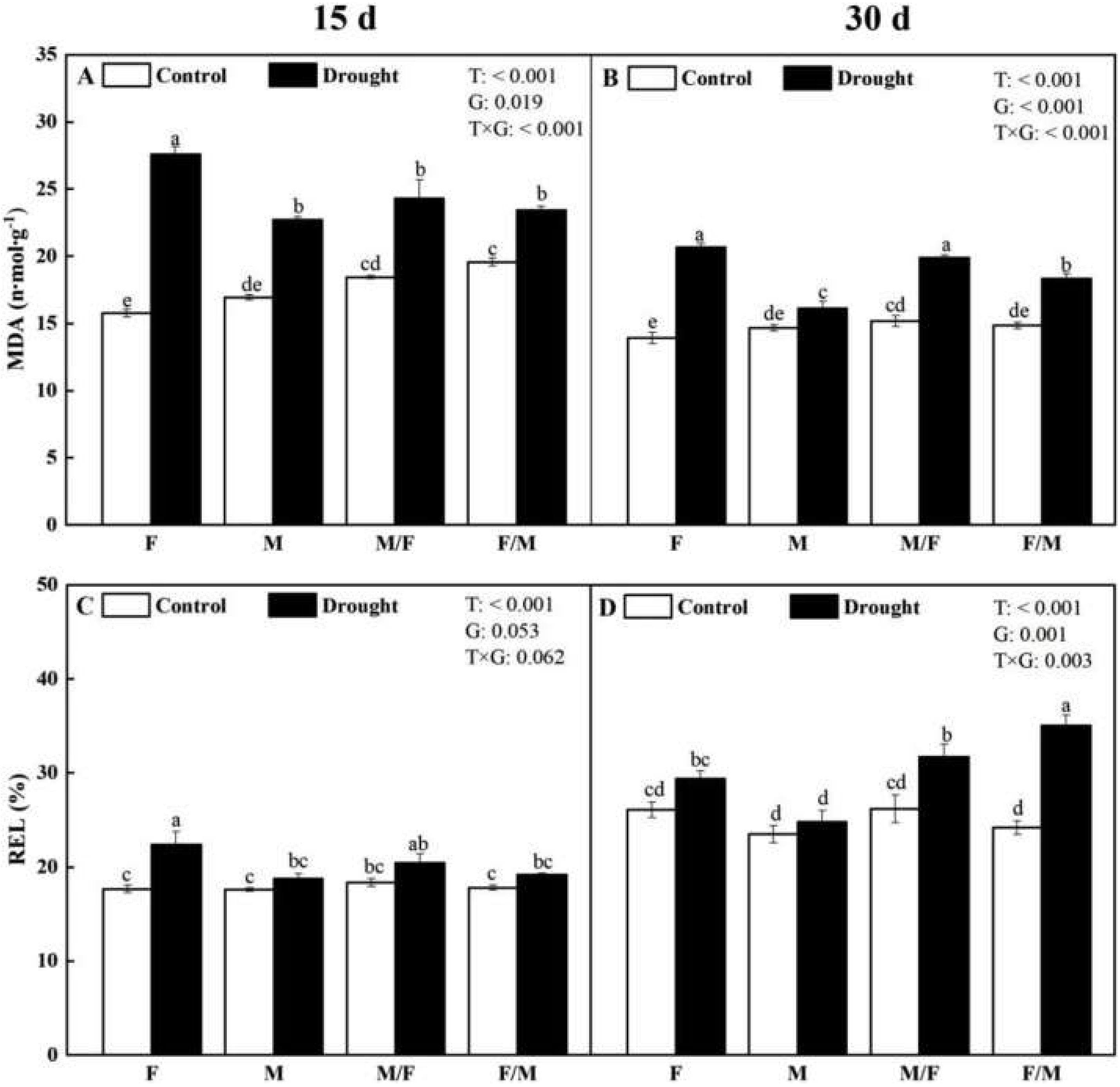

Fig. 7. MDA (A, B) and REL (C, D) in female genotype, *Populus euramericana* cv. "Nanlin895" (NL-895) (F), male genotype *Populus deltiodes* cv.*"*3412*"* (NL-3412) (M), and reciprocal grafted seedlings between male and female clones under water deficit stress. The values are shown as mean ± SE (n ≥ 10). Different letters indicate significant differences among treatments according to Duncan's test at a significance level of $p < 0.05$. M/F, male shoot scion and female rootstock; F/M, female shoot scion and male rootstock; Control, normal watering treatment (field water content equaling 80%); Drought, severe drought stress (field capacity of 30%); T, treatment (drought) effect; G, genotype effect; T × G, treatment (drought) × genotype effect.

4. Discussion

Previous studies have showed that scion growth was positively correlated with root hydraulic conductivity, which depended on rootstock (Zhang et al., 2016; Han et al., 2018). In the present study, we found that grafting with drought-tolerant male root could improve drought tolerance by decreasing less biomass, and increasing more WUE and antioxidant defense systems of grafted seedlings, which indicated that plants with male roots can absorb more soil water and maybe control leaf water loss efficiently to promote plant growth (Han et al., 2019).

As a rapid alternative tool to relatively slow breeding approaches in many plant species (Flores et al., 2010; Liu et al., 2016; Han et al., 2013), grafting method has been applied to protect plants against environmental stress and enhance plant's resistance to various biotic and abiotic stresses (Zhang et al., 2019). Rootstock can directly affect the phenological phase, nutritional development and production (Tecchio et al., 2020). In this study, most seedlings with female roots were more sensitive to water deficit compared to seedlings with male roots, which may be caused by drought tolerance characteristics of female roots. Females would allocate more resources for reproduction than males, resulting in lower growth rates and greater sensitivity to adverse environmental conditions than males (Han et al., 2013). This induced that a rootstock from a drought-tolerant genotype, i.e. the male clone in this case, was generally beneficial for plant growth, and M/F grafted with female roots were more sensitive to drought stress. Moreover, the differences in response to drought stress between the grafted and non-grafted seedlings indicated that the root system influences shoot growth, which might be caused by hormone delivery from the roots to the shoots that regulates shoot physiology (Santana-Vieira et al., 2016).

Water stress is an important environmental limiting factor for plant growth. Previous studies have found that plants would have a lower growth rate under drought (Wang et al., 2017; He et al., 2016), as detected in this study which was reflected in leaf traits and biomass. Water deficit could inhibit cell expansion and cell growth due to decreased leaf turgor. Decrease in the leaf length, leaf area, and biomass illustrated that plant growth was associated with the decline in the cell enlargement (Rawat et al., 2017). Drought stress on reciprocally grafted plants reduced most of the leaf traits significantly compared with non-grafted seedlings, which may be an adaptive response to improve water use efficiency. Furthermore, it was also found that delayed leaf wilting in grafted F/M seedlings under water deficit though no significances were observed on leaf area and biomass between grafted M/F and F/M. The difference in leaf traits between the grafted and non-grafted seedlings also indicated the root system plays an important role in improving drought tolerance (Han et al., 2019). Rootstock could result in higher shoot growth (base diameter) when grafted with scion (Kumar et al., 2017). However, drought stress reduced height of grafted seedlings significantly compared with non-grafted female seedling but increased basal diameter of M/F significantly compared with non-grafted male seedlings ($P < 0.05$; Fig. 2A, B, C, D). Combined with biomass allocation, one reason might be that plants would allocate relatively more biomass to their underground parts under water deficit conditions, thereby increasing the absorption and utilization of limited resources (Shipley and Meziane, 2002). Another reason might be that the scion also conferred vigor to the rootstock (Sun et al., 2020) and female rootstocks altered the secondary growth (stem thickness) ($P < 0.05$) rather than the primary growth (stem length) ($P > 0.05$) (Cookson and Ollat, 2013). It is evident the growth differences between females and males under well-watered conditions are predominantly linked to shoots, as higher plant height and biomass were observed in female seedlings.

Significant differences were found in gas exchange between grafted and non-grafted seedlings in this study. Moreover, these differences were observed in different gender rootstock-grafted seedlings, indicating that the gender effect of rootstock on response to water deficit stress. It seems that grafting altered the stomatal movement, especially under water deficit stress. In grafted seedlings, decreased WUE over non-grafted seedlings was due to a higher transpiration rate in comparison to non-grafted plants under water deficit stress. Rootstock-induced changes in stomatal development also influence leaf water conservation in grafted seedlings, because reduced transpiration relates to lower stomatal density in grafted plants (Liu et al., 2016). Importantly, Gs decreased more in M/F than in male seedlings under stress indicated that more stomatal closure was induced in M/F plants. The reason might be that male roots provide the ability to maintain water relationships and protective mechanisms. A reduction in Gs and Tr usually leads to a decrease or increase in WUE, depending on the severity of stress and plant species. In this study, grafting enhanced WUE more in F/M than in female seedlings under stress. The WUE differences between F/M and M/F are probably a sign for different responses to severe soil water deficit, which could be attribute to the rootstock effects (Peng et al., 2013; Han et al., 2013). These results revealed that grafting could affect the drought tolerance of plants, probably due to the stomatal factors that decrease photosynthesis which would induce the changes of root-to-shoot hydraulic and chemical signaling. Moreover, seedlings with female roots (F and M/F) showed more severe chloroplast damage than did seedlings with male roots (M and F/M) under similar drought stress conditions, suggesting that different graft combination could balance water uptake and loss in order to alleviate the detrimental effects of drought on net CO2 assimilation rate (Kumar et al., 2017).

Drought can damage cell membranes and increase membrane permeability, which can lead to lipid peroxidation caused by ROS. The active oxygen-scavenging systems of plants can protect cell membranes from damage (Jaleel et al., 2009). Drought stress induces remarkable increases in oxidative damage to all seedlings. SOD, POD and CAT activities of F/M showed greater increases under stress than those of M/F, which might indicate that the detoxification systems function more efficiently in F/M thus protecting plants from ROS toxicity under drought stress. MDA, the cytotoxic product of lipid peroxidation caused by ROS, is a well-known indicator of oxidative lipid damage caused by ROS under stress (Ayala et al., 2014). Seedlings with the female root displayed greater increases in the MDA contents compared to seedlings with male roots, indicating more severe damage in membrane lipids (Liao et al., 2017) in the former grafting combinations. Higher relative electrolyte leakage were observed in the grafted seedlings, which indicates that stress caused greater damage to their membrane system, suggesting that these plants exhibited lower drought tolerance capacity than the non-grafted plants. However, higher REL in grafted F/M seedlings at 30 d indicated that tolerance of male rootstock with female scion can also affect by shoot, possibly due to the role of abscisic acid in ROS scavenging activity and regulation of antioxidant reaction in response to drought (Liu et al., 2016). Increased H2O2 accumulation in shoot leaves could possibly play a signaling role in rapid stomatal closure (Liu et al., 2016).

In conclusion, our phenotypic and physiological data demonstrated that grafted seedlings with male roots can effectively alleviated the inhibition of growth induced by drought stress. Drought tolerance of grafted seedlings is primarily caused by the rootstock, although the scion also affects the plant. Nevertheless, the present findings clearly indicate the effect of drought-tolerant rootstock on the shoot. Further, a detailed study is required to elucidate the underlying biochemical and genetic mechanisms which are responsible for differential response of grafted combinations.

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Author contributions

Meihua Liu designed the experiments. Shengxian Chen analyzed the data. Lita Yi and Fei Yu provided guidance in the methodology and use of software. Meihua Liu and Shengxian Chen wrote the draft. Helena Korpelainen revised the draft and provided language help. Meihua Liu had the overall responsibility for the study.

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.

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