The impacts of space mutation and host genotype on endophyte role in host perennial ryegrass stress tolerance

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Research Article

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Abstract

Aims This study reveal if host genotype, endophyte or space mutation have effects on host stress resistance and select the best performance genotype.

Methods Stress tolerance of 8 different perennial ryegrass genotypes including endophyte infected (E+) and endophyte free (E-) with and without space mutation were evaluated. Four different treatments were established which included control {CK, 45% of relatively soil moisture content (RSWC)}, drought stress (D, 15% RSWC), salt stress (S, 250 mmol NaCl with 45% RSWC) and drought combined with salt stress (DS, 250 mmol NaCl with 15% RSWC).

Results The results showed that stress treatments significantly inhibited (P< 0.05) the growth of perennial ryegrass as plant height, tiller numbers and biomass of most plants significantly decreased (P<0.05). Under both control and stress treatments, host genotypes had significant (P<0.05) effects on plant growth, contents of phytohormones, ion and nutrient elements. However, there is no consistent performance for each host genotype. For plants both with and without space mutation, endophyte promoted host growth, regulated the rational distribution of ion content and nutrient elements in host plant. The space mutation had effects on plants performance as there was significant difference (P< 0.05) between mutation and without mutant for all of the tested indices except with plant height, aboveground biomass, CTK contents, underground Na^+ and C/P, $S_{K, Na}$, aboveground C.

Conclusions The effects of host genotype on plant performance was much more pronounced and far greater than that of endophyte infection and mutation. Endophyte had effects on more indices of plants without mutant which suggest mutation may hide some effects of endophyte as its strong effects.

Introduction

Perennial ryegrass (*Lolium perenne*) is a globally important forage and turf grass species due to its desirable agronomic performance in temperate climates. Ryegrass has remarkable characteristics that makes it a dominant species, of which the most outstanding are high herbage yield and quality, favorable palatability and high grazing persistence (Yamada et al. 2005; Giraldo et al. 2018). Extensive studies have confirmed that *Epichloë festucae* var. *lolii* endophyte is an essential component of perennial ryegrass cultivation systems, due to the capacity to enhance agronomic performance (Latch et al. 1984; Leuchtmann et al. 2014). *E. festucae* var. *lolii* mutualistically interact with perennial ryegrass by providing major fitness enhancements and protection from both biotic and abiotic stresses (Johnson et al. 2013; Young et al. 2013; Ross 2016). Endophytes confer distinct agronomic advantages, but can be detrimental to the health and production of grazing mammals as herbivore toxicity effects associated with the production of specific alkaloids such as lolitrem B and ergovaline (Siegel et al. 1990; Easton et al. 2002). Therefore, selection and transfer of endophytes that were less toxic to livestock, whilst maintaining certain advantageous traits have been successfully developed and marketed in cultivars (Johnson et al. 2013; Young et al. 2013). However, most of commercial perennial ryegrass cultivars widely used in China

were imported from overseas. Multiple approaches have been implemented to breed new perennial ryegrass varieties of domestic intellectual property and promote seeds production localization. Space mutation has been applied to plant breeding in the past 30 years in China and a number of new cultivars or selections of rice, wheat, maize, green pepper and watermelon were developed by this method (Liu et al. 2007; Paran et al. 2007).

Our previous studies have shown that perennial ryegrass cultivar ‘Pinnacle’ had high resistance to leaf spot disease (Tian et al. 2008; Ma et al. 2015) and tolerance to drought (Li et al. 2016) in Lanzhou, China, which suggest that this cultivar had potential to be widely adopted for cultivation in the local region. In order to overcome some of limitations of traditional introgression and selective breeding, some of seeds were treated with space mutation and further mutant germplasm resources were established (Tian et al. unpublished data). We measured some growth parameters of individual plants and selected some plants with excellent performance for further evaluation and utilization.

In the present study, the further stress resistance evaluation of these selected individual plants was conducted. The objectives of the present study were to 1) assess if these high growth performance perennial ryegrass individuals have strong stress resistance; 2) reveal if the endophyte or space mutation have effects on host stress resistance; 3) understand their physiological factors underlying stress tolerance for the breeding of stress-tolerant cultivars.

Materials And Methods

Plants materials

Seeds of perennial ryegrass were supplied by Lanzhou university which have been screen for infection rates in 2014 and 2015 at the Yuzhong Experimental Station of Lanzhou University (104°39' E, 35°89 N, Altitude 1653 m), Gansu Province, China (Chen et al. 2020 a, b). The seeds from endophyte infected (E+) and endophyte free (E-) subpopulations were marked and stored at 4 °C. Two hundred seeds per E+ and E- population were carried by “Shenzhou11” in Oct, 2016. These seeds were marked as space mutation (ME+ and ME-) and stored at 4 °C. In April 16, 2017, these space mutation seeds and same seeds without space mutation (UE+ and UE-) were planted experimental field blocks at the Yuzhong Experimental Station of Lanzhou University. The growth and morphology of all the plants were evaluated to selected the better performance plants (Tian et al. unpublished data). The seeds were harvested from each individual plants and then stored at 4 °C.

Experimental design

In May 2019, the well filled, healthy-looking E+ and E- seeds harvested from individual with better performance were planted in plastic trays (30 cm × 25 cm × 8 cm) filled with 1.5 kg soil (commercial fine sandy soil, Lanzhou) which had been sterilized in an oven at 130℃ for 30 min. Five rows with 10 seeds were planted per tray at a depth of 1 cm. The seeds included 2 genotypes from E+ with mutant (ME+1, ME+2), 2 genotypes from E- with mutant (ME-1, ME-2), 2 genotypes from E+ without mutant (UE+1, UE+2)

and 2 genotypes from E- without mutant (UE-1, UE-2). Two trays per genotype were prepared and placed in a temperature controlled greenhouse (18℃ - 24℃) with 10 h of illumination per day in Yuzhong campus of Lanzhou University. After plants had 4 tillers, endophyte viability in each seedling was determined by microscopic examination of the host leaf sheath pieces after they had been stained with aniline blue (Nan 1996). The seedlings germinated from E+ seeds with characteristic longitudinally-orientated hyphae of the endophyte were marked as E+ and the seedlings germinated from E- seeds without hyphae were marked as E-. The marker seedlings were transplanted into round pots (upper diameter-13 cm × lower diameter-10 cm × height-11 cm) containing the same amount of media (sterilized commercial vermiculite and black soil in a w/w ratio 1:3). Each pot had only one similar growth seedling and equal initial water treatment. After one-month stabilization with same irrigation, four different treatments were established, the pots were weighted and watered to maintain the appropriated relatively soil moisture content (RSMC) and the same amount volume 250mmol NaCl was irrigated before treatment to keep the conditions as following: CK (45% RSMC), drought stress treatment (D, 15% RSMC), salt stress treatment (S, 45% RSMC with 250mmol NaCl) and drought combined with salt treatment (DS,15% RSMC with 250mmol NaCl). Each treatment has 6 replicates per genotype which were randomly placed in greenhouse maintained at a constant condition (temperature: 25 ± 2 ℃, humidity: 42 ± 5%). After another month growth under treatment, the plants were destructively harvest for evaluation.

Experimental evaluations

After 28 days growth, 2 gram fresh leaves were collected from each plant for gibberellin (GA_3), indole-3-acetic acid (IAA), cytokinins (CTK), salicylic acid (SA) and abscisic acid (ABA) contents test using enzyme-linked immunosorbent assay (Danshi biology, Shanghai, China).

After 28 days growth, plant height and tiller number of each plant were recorded. The whole plants were then carefully removed from pots, washed with distilled water and dried on a filter paper. All harvested plants were separated into roots and shoots and their fresh weight recorded. Dry weight was obtained after oven-drying the tissue at 60℃ until a constant weight was reached. After weighting, the plant materials were ground twice using a mixer mill (Retch 400MM, German) at 30 Hz for 2 min for analysis of ions contents and nutrient elements.

Na^+, K^+, Ca^{2+} ions contents were analysed by using atomic absorption spectrometry (M6AA system, Thermo, USA) after mineralization in mixture of acids (Hanway and Heidel 1952). Based on these results, the ratio of Na^+/ K^+ and $S_{K, Na}$ were calculated.

$S_{K, Na}$ =(aboveground K^+/ Na^+)/(underground K^+/ Na^+) (Flowers and Yeo 1988).

Carbon (TC) contents were determined with K_2CrO_7 oxidation method (Tanveer et al. 2014). The total nitrogen (TN) and total phosphorus (TP) contents were determined following digestion with sulfuric acid (H_2SO_4) at a temperature of 420 °C and the concentrations in the digested solutions were determined by flow injection analysis, using a FIAstar 5000 Analyzer (FOSS Analytical, Denmark) (Xia et al. 2018).

Statistical analyses

Statistical data analysis was performed with SPSS Inc. (Released 2009. PASW Statistics for Windows, Version 25.0. Chicago: SPSS Inc). Means are reported with their standard errors. Univariate analysis of general linear models was employed to estimate the effects of single factor and their interaction on indices of perennial ryegrass in the present study (Table S1). Significant difference between single factors (stress treatment, host genotype, endophyte and mutation) were assessed by Least Significant Difference (LSD) tested at $p<0.05$ and generated from one-way analysis of variance (ANOVA) based on the separated dataset. Statistical significance was defined at the 95% confidence level.

Results

Plant growth

Stress treatments had significant inhibition (*P<0.05*) on plant height of ME-2, UE+1, UE+2 and UE-1 (Fig.1.). Host genotype also had significant effects (*P<0.05*) on plant height under control and drought stress. Host genotype performance varied without consistence under these two treatments. Endophyte only had significant effects on plant height of plants without mutant as that of E+ plants was significantly higher (*P<0.05*) than that of E- plants only under control. Mutation treatment had no effects on plant height.

Stress treatments had significant inhibition effects (*P<0.05*) on tiller numbers of these 8 genotypes (Fig.2.). Host genotype also had significant effects (*P<0.05*) on tiller numbers under these four treatments. Endophyte had significant effects (*P<0.05*) on tiller number. For plants with mutant, tiller numbers of E+ plants were significantly higher (*P<0.05*) than those of E- plants under salt stress; for plants without mutant, those of E+ plants were significantly higher (*P<0.05*) than those of E- plants under these four treatments. Mutation treatment had significant effects on tiller numbers as those of plants with mutant were significantly higher (*P<0.05*) than those of plants without mutant under drought stress and salt stress.

Stress treatments had significant inhibition effects (*P<0.05*) on aboveground biomass of these 8 genotypes (Fig.3.). Host genotype also had significant effects (*P<0.05*) on aboveground biomass as there was significant difference between these 8 host genotypes whose performance varied without consistence under these four treatments except with that UE+2 always had highest aboveground biomass. Endophyte had significant effects (*P<0.05*) on aboveground biomass. For plants with mutant, aboveground biomass of E+ plants were significantly lower (*P<0.05*) than those of E- plants under salt stress; for plants without mutant, those of E+ plants were significantly higher (*P<0.05*) than those of E- plants under salt and drought with salt stress. Mutation treatment had no effects on aboveground biomass.

Stress treatments had significant inhibition effects (*P<0.05*) on underground biomass of ME-1, ME-2, UE+1, UE+2 and UE-1(Fig.4.). Host genotype also had significant effects (*P<0.05*) on underground

biomass as there was significant difference between these 8 host genotypes under these four treatments whose performance varied without consistence under these four treatments except with that ME+1 and ME-1 always had higher (*P<0.05*) aboveground biomass. Endophyte had effects on underground biomass as that of E+ plants with mutant was significantly lower (*P<0.05*) than that of E- plants with mutant under control. Mutation treatment also had significant effects on underground biomass as those of plants with mutant were significantly higher (*P<0.05*) than those of plants without mutant under drought stress and drought with salt stress.

Phytohormones

Stress treatments had significant effects on IAA contents of 7 genotypes except with ME-2 (Fig.5.). Stress treatments significantly improved (*P<0.05*) IAA contents of ME+1, ME-1 and UE-1 whereas significantly reduced (*P<0.05*) those of ME+2 and UE-2. Host genotype also had significant effects (*P<0.05*) on IAA contents as there was significant difference between these 8 host genotypes whose performance varied without consistence under these four treatments except with that UE-1 always had highest (*P<0.05*) IAA contents. Endophyte had no effects on IAA contents. Mutation treatment had significant effects on IAA contents as those of plants with mutant were significantly lower (*P<0.05*) than those of plants without mutant under drought stress and salt stress.

Stress treatments had significantly different effects on ABA contents of these 8 genotypes (Fig.6.). Stress treatments significantly improved (*P<0.05*) ABA contents of ME-2 whereas significantly reduced (*P<0.05*) those of ME+1, UE+1 and UE+2. However, some of stress treatments significantly improved (*P<0.05*) whereas some of stress treatments significantly reduced (*P<0.05*) ABA contents of ME+2, ME-1, UE-1 and UE-2. Host genotypes also had significant effects (*P<0.05*) on ABA contents as there was significant difference between these 8 host genotypes whose performance varied without consistence under these four treatments. Endophyte had no effects on ABA contents. Mutation treatment had significant effects on ABA contents as those of plants with mutant were significantly lower (*P<0.05*) than those of plants without mutant under control and salt stress.

Stress treatments had significantly different effects on CTK contents of 7 genotypes except with UE+2 (Fig.7.). Some of stress treatments significantly improved (*P<0.05*) CTK contents of ME+1, ME-1, UE+1 and UE-2 whereas significantly reduced (*P<0.05*) those of ME-2 and UE-1. However, salt stress significantly improved (*P<0.05*) whereas drought stress and drought with salt stress significantly reduced (*P<0.05*) CTK contents of ME+2. Host genotype also had significant effects (*P<0.05*) on CTK contents as there was significant difference between these 8 host genotypes whose performance varied without consistence under these four treatments except with that ME+1 always had highest (*P<0.05*) CTK contents. Endophyte had effects on CTK contents as there was significant difference (*P<0.05*) between E+ plants and E- plants without mutant under salt stress and drought with salt stress. Mutation treatment had no effects on CTK contents.

Stress treatments had different effects on GA contents of 7 genotypes except with UE-1 (Fig.8.). Stress treatments significantly improved (*P<0.05*) GA contents of ME-1 and UE-2 whereas significantly reduced

(*P<0.05*) those of UE+2. However, some of stress treatments significantly improved (*P<0.05*) whereas some of stress treatments significantly reduced (*P<0.05*) GA contents of ME+1, ME-2, and UE+1. Host genotype also had significant effects (*P<0.05*) on GA contents as there was significant difference between these 8 host genotypes under these four treatments whose performance varied without consistence under these four treatments except with that UE-2 had highest (*P<0.05*) GA contents under stress. Endophyte had effects on GA contents. For plants with mutant, GA contents of E+ plants were significantly higher (*P<0.05*) than those of E- plants under control; for plants without mutant, those of E+ plants were significantly lower (*P<0.05*) than those of E- plants under salt and drought with salt stress. Mutation treatment had significant effects on GA contents as those of plants with mutant were significantly lower (*P<0.05*) than those of plants without mutant under control, drought stress and salt stress.

Stress treatments had significant effects on SA contents of these 8 genotypes (Fig.9.). Stress treatments significantly improved (*P<0.05*) SA contents of UE-2 whereas significantly reduced (*P<0.05*) those of ME-1, ME-2 and UE+2. However, some of stress treatments significantly improved (*P<0.05*) whereas some of stress treatments significantly reduced (*P<0.05*) SA contents of ME+1, ME+2, UE+1 and UE-1. Host genotype also had significant effects (*P<0.05*) on SA contents as there was significant difference between these 8 host genotypes whose performance varied without consistence under these four treatments. Endophyte had effects on SA contents. For plants with mutant, SA contents of E+ plants were significantly higher (*P<0.05*) than those of E- plants under drought stress and salt stress; for plants without mutant, those of E+ plants were significantly different (*P<0.05*) with those of E- plants under control, drought stress and salt stress. Mutation treatment had significant effects on SA contents as those of plants with mutant were significantly lower (*P<0.05*) than those of plants without mutant under control, drought stress and drought with salt stress.

ion contents

Stress treatments had significant effects (*P<0.05*) on aboveground Na^+ contents of these 8 genotypes (Fig.10.). Stress treatments significantly improved (*P<0.05*) Na^+ contents of 6 genotypes except with ME-1 and UE-1. However, salt stress and drought with salt stress significantly improved (*P<0.05*) whereas drought stress significantly reduced (*P<0.05*) Na^+ contents of ME-1 and UE-1. Host genotype also had significant effects (*P<0.05*) on aboveground Na^+ ion contents as there was significant difference between these 8 host genotypes whose performance varied without consistence under these four treatments. Endophyte had effects on aboveground Na^+ ion contents. For plants with mutant, Na^+ contents of E+ plants were significantly higher (*P<0.05*) than those of E- plants under control; for plants without mutant, those of E+ plants were significantly higher (*P<0.05*) than those of E- plants under drought stress and drought with salt stress. Mutation treatment had significant effects on aboveground Na^+ contents as those of plants with mutant were significantly different (*P<0.05*) with those of plants without mutant under control, drought stress and salt stress.

Stress treatments had significantly different effects on underground Na^+ contents of 7 genotypes except with ME-2 (Fig.11.). Stress treatments significantly improved (*P<0.05*) Na^+ contents of ME+1, UE+1, UE-2 whereas significantly reduced (*P<0.05*) those of ME+2, UE+2, UE-2. However, salt stress significantly improved (*P<0.05*) whereas drought with stress significantly reduced (*P<0.05*) Na^+ contents of ME-1. Host genotype also had significant effects (*P<0.05*) on underground Na^+ contents as there was significant difference between these 8 host genotypes whose performance varied without consistence under these four treatments. Endophyte had effects on underground Na^+ contents. For plants with mutant, those of E+ plants were significantly higher (*P<0.05*) than those of E- plants under drought with salt stress; for plants without mutant, those of E+ plants were significantly lower (*P<0.05*) than those of E- plants under control. Mutation treatment had no significant effects on underground Na^+ ion contents.

Stress treatments significantly reduced (*P<0.05*) aboveground K^+ contents of these 8 genotypes (Fig.12.). Host genotypes also had significant effects (*P<0.05*) on aboveground K^+ contents as there was significant difference between these 8 host genotypes whose performance varied without consistence under these four treatments except with that ME+2 always had highest (*P<0.05*) contents. Endophyte had little effects on aboveground K^+ contents as those of E+ plants without mutant were significantly higher (*P<0.05*) than those of E- plants without mutant under drought stress and drought with salt stress. Mutation treatment had significant effects on aboveground K^+ contents as those of plants with mutant were significantly higher (*P<0.05*) than those of plants without mutant under control and salt stress(*P<0.05*).

Stress treatments significantly improved (*P<0.05*) underground K^+ contents of ME+1, UE+1 and UE-1 whereas significantly reduced (*P<0.05*) those of ME+2, ME-1, ME-2, UE+2 and UE-2 (Fig.13.). Host genotype also had significant effects (*P<0.05*) on underground K^+ ion contents as there was significant difference between these 8 host genotypes whose performance varied without consistence under these four treatments. Endophyte had little effects on underground K^+ ion contents as those of E+ plants with mutant were significantly higher (*P<0.05*) than those of E- plants with mutant under drought with salt stress. Mutation treatment had significant effects on underground K^+ contents as those of plants with mutant were significantly higher (*P<0.05*) than those of plants without mutant under control.

Stress treatments significantly improved (*P<0.05*) aboveground Ca^{2+} contents of ME+2 and ME-1 whereas significantly reduced (*P<0.05*) those of UE+1 (Fig.14.). Host genotype also had significant effects (*P<0.05*) on aboveground Ca^{2+} contents as there was significant difference between these 8 host genotypes whose performance varied without consistence under these four treatments. Endophyte had effects on aboveground Ca^{2+} contents. For plants with mutant, those of E+ plants were significantly lower (*P<0.05*) than those of E- plants under control and salt stress; for plants without mutant, those of E+ plants were significantly higher (*P<0.05*) than those of E- plants under control. Mutation treatment had significant effects on aboveground Ca^{2+} contents as those of plants with mutant were significantly different (*P<0.05*) with those of plants without mutant under control and drought stress.

Stress treatments significantly improved (*P<0.05*) underground Ca^{2+} ion contents of UE+1 whereas significantly reduced (*P<0.05*) those of ME+2 and ME-1. However, drought stress significantly reduced (*P<0.05*) whereas the other two stress significantly improved (*P<0.05*) Ca^{2+} ion contents of UE-2 (Fig.15.). Host genotype had significant effects (*P<0.05*) on underground Ca^{2+} ion contents as there was significant difference between these 8 host genotypes whose performance varied without consistence under these four treatments. Endophyte had significant effects on underground Ca^{2+} ion contents. For mutant plants, those of E+ plants were significantly higher (*P<0.05*) than those of E- plants under three stress treatments; for plants without mutant, those of E+ plants were significantly higher (*P<0.05*) than those of E- plants under control and drought stress. Mutation treatment had significant effects on underground Ca^{2+} ion contents as those of plants with mutant were significantly higher (*P<0.05*) than those of plants without mutant under control (*P<0.05*).

Salt and drought with salt stress treatments significantly improved (*P<0.05*) aboveground ratio of Na^+/ K^+ of these 8 genotypes (Fig.16.). Host genotype also had significant effects (*P<0.05*) on aboveground ratio of Na^+/ K^+ as there was significant difference between these 8 host genotypes whose performance varied without consistence under these four treatments except with that UE-1 was always highest (*P<0.05*). Endophyte had little effects on aboveground ratio of Na^+/ K^+. For plants with mutant, that of E+ plants was significantly higher (*P<0.05*) than that of E- plants under control; for plants without mutant, that of E+ plants was significantly higher (*P<0.05*) than that of E- plants under salt stress. Mutation treatment had significant effects on aboveground ratio of Na^+/ K^+ as that of plants with mutant were significantly lower (*P<0.05*) than that of plants without mutant under control and drought stress.

Stress treatments significantly improved (*P<0.05*) underground ratio of Na^+/ K^+ of ME+1, ME-1, ME-2 and UE+1 whereas significantly reduced (*P<0.05*) that of UE-2 (Fig.17.). Host genotype also had significant effects (*P<0.05*) on underground ratio of Na^+/ K^+ as there was significant difference between these 8 host genotypes whose performance varied without consistence under these four treatments. Endophyte had little effects on underground ratio of Na^+/ K^+ as that of E+ plants without mutant was significantly lower (*P<0.05*) than those of E- plants without mutant under control. Mutation treatment had significant effects on underground ratio of Na^+/ K^+ as those of plants with mutant were significantly lower (*P<0.05*) than those of plants without mutant under control and drought stress.

Stress treatments significantly improved (*P<0.05*) $S_{K, Na}$ of these 8 genotypes (Fig.18.). Host genotype also had significant effects (*P<0.05*) on $S_{K, Na}$ as there was significant difference between these 8 host genotypes whose performance varied without consistence under these four treatments except with that UE+2 always had highest $S_{K, Na}$ (*P<0.05*). Endophyte had little effects on $S_{K, Na}$ as that of E+ plants without mutant was significantly higher (*P<0.05*) than that of E- plants without mutant under control. Mutation treatment had no significant effects on $S_{K, Na}$.

Elements

Stress treatments had significant effects (*P<0.05*) on aboveground C contents of these 8 genotypes (Fig.19.). Stress treatments significantly reduced (*P<0.05*) C contents of ME+1, ME-1, UE+2 and UE-2. However, some of stress treatments significantly improved (*P<0.05*) whereas some of stress treatments significantly reduced (*P<0.05*) C contents of ME+2, ME-2, UE+1 and UE-1. Host genotype also had significant effects (*P<0.05*) on aboveground C contents as there was significant difference between these 8 host genotypes whose performance varied without consistence under these four treatments. Endophyte had significant effects on aboveground C contents. For plant with mutant, those of E+ plants were significantly lower (*P<0.05*) than those of E- plants under drought with salt stress; for plants without mutant, those of E+ plants were significantly lower (*P<0.05*) than those of E- plants under control, drought stress and drought with salt stress. Mutation treatment had no significant effects on aboveground C contents.

Stress treatments had significant effects (*P<0.05*) on underground C contents of these 8 genotypes (Fig.20.). Some of stress treatments significantly improved (*P<0.05*) C contents of ME+1, UE+1 and UE-2 whereas significantly reduced (*P<0.05*) those of ME+2, ME-1, UE+2 and UE-1. However, drought stress significantly reduced (*P<0.05*) whereas the other two stresses significantly improved (*P<0.05*) C contents of ME-2. Host genotype also had significant effects (*P<0.05*) on underground C contents as there was significant difference between these 8 host genotypes whose performance varied without consistence under these four treatments. Endophyte had effects on underground C contents. For plants with mutant, those of E+ plants were significantly higher (*P<0.05*) than those of E- plants under stress treatments; for plants without mutant, those of E+ plants were significantly higher (*P<0.05*) than that of E- plants without mutant under control and drought with salt stress. Mutation treatment had significant effects on underground C contents as those of plants with mutant were significantly higher (*P<0.05*) than those of plants without mutant.

Stress treatments significantly improved (*P<0.05*) aboveground N contents of 7 genotypes except with UE-1 (Fig.21.). Host genotypes also had significant effects (*P<0.05*) on aboveground N contents as there was significant difference between these 8 host genotypes whose performance varied without consistence under these four treatments except with that ME+2 always had highest N contents (*P<0.05*). Endophyte had little effects on aboveground N contents as those of E+ plants without mutant were significantly higher (*P<0.05*) than those of E- plants without mutant under drought stress. Mutation treatment had significant effects on aboveground N contents as those of plants with mutant were significantly higher (*P<0.05*) than those of plants without mutant under salt stress and drought with salt stress.

Stress treatments significantly reduced (*P<0.05*) underground N contents of UE+1 and UE+2 whereas significantly increased (*P<0.05*) those of the other 5 genotypes except with UE-2 (Fig.22.). Host genotype also had significant effects (*P<0.05*) on underground N contents as there was significant difference between these 8 host genotypes whose performance varied without consistence under these four treatments. Endophyte had effects on underground N contents. For plants with mutant, those of E+ plants were significantly lower (*P<0.05*) than those of E- plants under control; for plants without mutant, those of

E+ plants were significantly different (*P<0.05*) than those of E- plants under control and salt stress. Mutation treatment had significant effects on underground N contents as those of plants with mutant were significantly lower (*P<0.05*) than those of plants without mutant under control and drought stress, vice versa under the other two stresses.

Stress treatments significantly reduced (*P<0.05*) aboveground P contents of these 8 genotypes (Fig.23.). Host genotypes also had significant effects (*P<0.05*) on aboveground P contents as there was significant difference between these 8 host genotypes whose performance varied without consistence under these four treatments. Endophyte had effects on aboveground P contents as those of E+ plants without mutant were significantly higher (*P<0.05*) than those of E- plants without mutant under drought with salt stress. Mutation treatment had significant effects on aboveground P contents as those of plants with mutant were significantly lower (*P<0.05*) than those of plants without mutant under drought with salt stress.

Stress treatments significantly improved (*P<0.05*) underground P contents of ME+2 and UE-1 whereas significantly reduced (*P<0.05*) those of ME+1, ME-1 and UE+1 (Fig.24.). Host genotype also had significant effects (*P<0.05*) on underground P contents as there was significant difference between these 8 host genotypes whose performance varied without consistence under these four treatments. Endophyte had effects on underground P contents. For plants with mutant, those of E+ plants were significantly different (*P<0.05*) with those of E- plants under drought stress and salt stress; for plants without mutant, those of E+ plants were significantly lower (*P<0.05*) than those of E- plants under drought stress. Mutation treatment had significant effects on underground P contents as those of plants with mutant were significantly higher (*P<0.05*) than those of plants without mutant under salt stress.

Some of stress treatments significantly reduced (*P<0.05*) aboveground C/N of these 8 genotypes (Fig.25.). Host genotype also had significant effects (*P<0.05*) on aboveground C/N as there was significant difference between these 8 host genotypes whose performance varied without consistence under these four treatments. Endophyte had little effects on aboveground C/N as that of E+ plants without mutant was significantly lower (*P<0.05*) than that of E- plants without mutant under drought stress. Mutation treatment had significant effects on aboveground C/N as that of plants with mutant were significantly lower (*P<0.05*) than those of plants without mutant under salt stress and drought with salt stress (*P<0.05*).

Stress treatments significantly improved (*P<0.05*) underground C/N of UE+1, UE+2 and UE-2 whereas significantly reduced (*P<0.05*) that of ME+1, ME+2, ME-1, ME-2 and UE-1 (Fig.26.). Host genotypes also had significant effects (*P<0.05*) on underground C/N as there was significant difference between these 8 host genotypes whose performance varied without consistence under these four treatments. Endophyte had significant effects on underground C/N. For mutant plants, that of E+ plants was significantly different (*P<0.05*) with that of E- plants under control, drought stress and salt stress; for plants without mutant, that of E+ plants was significantly higher (*P<0.05*) than that of E- plants under drought with salt stress. Mutation treatment had significant effects on underground C/N as that of plants with mutant were

significantly higher (*P<0.05*) than those of plants without mutant under control, drought stress and drought with salt stress.

Some of stress treatments significantly improved (*P<0.05*) aboveground C/P of these 8 genotypes (Fig.27.). Host genotype had significant effects (*P<0.05*) on aboveground C/P as there was significant difference between these 8 host genotypes whose performance varied without consistence under these four treatments. Endophyte had no effects on aboveground C/P. Mutation treatment had significant effects on aboveground C/P as that of plants with mutant were significantly different (*P<0.05*) than that of plants without mutant under drought stress and drought with salt stress.

Some of stress treatments significantly improved (*P<0.05*) underground C/P of ME+1 and UE+1 whereas significantly reduced (*P<0.05*) that of ME+2, UE+2 and UE-1 (Fig.28.). Host genotype also had significant effects (*P<0.05*) on underground C/P as there was significant difference between these 8 host genotypes whose performance varied without consistence under these four treatments. Endophyte had significant effects on underground C/P. For plants with mutant, underground C/P of E+ plants was significantly different (*P<0.05*) with that of E- under control, drought stress and salt stress; for plants without mutant, that of E+ plants was significantly higher (*P<0.05*) than that of E- plants under drought stress. Mutation treatment had no significant effects on underground C/P.

Some of stress treatments significantly improved (*P<0.05*) aboveground N/P of these 8 genotypes (Fig.29.). Host genotype also had significant effects (*P<0.05*) on aboveground N/P as there was significant difference between these 8 host genotypes whose performance varied without consistence under these four treatments except with that ME+2 always had highest N/P. Endophyte had no significant effects on aboveground N/P. Mutation treatment had significant effects on aboveground N/P as those of plants with mutant were significantly higher (*P<0.05*) than those of plants without mutant under drought with salt stress.

Some of stress treatments significantly improved (*P<0.05*) underground N/P of ME+1, ME-1, UE+1 (Fig.30.). Host genotypes had significant effects (*P<0.05*) on underground N/P as there was significant difference between these 8 host genotypes whose performance varied without consistence under these four treatments. Endophyte had little effects on underground N/P as that of E+ plants without mutant was significantly different (*P<0.05*) with that of E- plants without mutant under drought stress and salt stress. Mutation treatment had significant effects on underground N/P as those of plants with mutant were significantly lower (*P<0.05*) than those of plants without mutant under control and drought stress(*P<0.05*).

Discussion

The stress treatments in the present study had similar inhibitions on plant growth which confirmed that both water deficiency and salt additive can bring damages to plant growth and physiology which lead to plant development restriction (Razmjoo 2008). Unexpectedly, the inhibition of the two combined drought and salt stress did not seem more serious than the drought or salt stress alone. Possible, the water

deficient have some inhibition to salt damages. Perennial ryegrass may have some mechanism to adjust its response to this combined abiotic stress.

Eight host genotypes were included and the effect of host genotype on plant performance and stress tolerance was far greater than that of endophyte infection and mutation. The influence of genotype on all recorded variables was much more pronounced. As perennial ryegrass is a cross-pollinating and self-incompatible plant species, large levels of genetic variation arise between individuals from the same population or cultivar (Wang et al. 2009). The evolutionary response of a host population to both biotic and abiotic environmental factors will depend on potential interactive effects of the host genotype and endophyte infection. Many researches have noted that endophyte-mediated effects on grasses are highly contingent on environmental conditions and host (and endophyte) genotype (Saikkonen et al. 1998; Cheplick et al. 2003; Cheplick 2004; Hesse et al. 2004). Genotypic variation in the response of host grasses to endophyte infection has been described in both *Festuca arundinacea* (Belesky et al. 1987; Elbersen et al. 1996; Marks et al. 1996) and *L. perenne* (Cheplick 1997, 1998; Cheplick et al. 2000). However, even though genotypes varied greatly in growth and physiology, there were few interactions between genotype and endophyte. The complex interaction between endophyte and host genotype may lead to these different performances of each individual. Increasing dry matter yield and stress tolerance of perennial ryegrass is of significant importance for perennial ryegrass breeding programs. These interactions suggested that the further germplasm selection process will be independent of whether individuals are infected by *E. festucae* var. *lolii*.

Some research indicated that the effects of endophyte on the growth of perennial ryegrass may be both positive and negative, depending on a combination for biotic and abiotic factors, as well as the interaction between the host and endophyte genotypes (Marks et al. 1991; Cheplick 1997, 1998). In the present study, *E. festucae* var. *lolii* improved host plant growth and stress tolerance. For genotypes with space mutation under some of treatments, there were significant difference ($P < 0.05$) between E+ plants and corresponding E- plants for tiller number, biomass, GA and SA contents, Na^+, Ca^{2+}, C contents, aboveground Na^+/K^+, underground K^+, N, P contents, C/N and C/P. For genotypes without space mutation under some of stress treatments, there were significant difference ($P < 0.05$) between E+ plants and corresponding E- plants for plant height, tiller number, aboveground biomass, CTK, GA and SA contents, Na^+, Ca^{2+}, Na^+/K^+, C, N, P contents, $S_{K, Na}$, C/N, aboveground K^+ and underground C/P and N/P. The results showed that, for plants both with and without space mutation, endophyte promoted host growth, regulated the rational distribution of ion content and nutrient elements in the host plant. These research suggested that endophyte plays some effects on host response to stress especially drought and salt stress. Endophyte had effects on more indices of plants without mutant which suggest mutation may hide some effects of endophyte as its effects.

Endophytes are thought to improve host tolerance to stress through a variety of morphological and physiological adaptations and adjustments, including promoting host growth and photosynthesis, increasing the levels of beneficial metabolites, activating antioxidant systems to scavenge ROS, and modulating plant growth phytohormones, improving nutrient uptake and maintains ionic homeostasis

(Malinowski et al. 2000, 2019; Song et al. 2015; Hume et al. 2016; Nagabhyru et al. 2013). In the present study, stress treatments did not have consistent effects on contents of IAA, ABA, CTK, GA_3 and SA. These contents significantly increased (P< 0.05) in some genotypes and significantly decreased (P< 0.05) in some genotypes. These changes of endogenous hormones in plants under different conditions confirmed that the plants utilize hormones during stress response. Phytohormones are very important signal molecules related to plant growth, physiological and developmental processes (Aaron et al. 2009). IAA is the most common auxin produced by plants, and its concentration is key in regulation of plant growth and development (Müller 2003). ABA is known to induce stomatal closure as a water conserving response in which plants benefit in the short term by reducing water loss via transpiration (Lemichez et al. 2001). CTK is a key element for improving grain yield by affecting the source/sink transition (Peleg et al. 2011). GA is a vital plant growth regulator, which has an important role in seed germination growth of floral organs, and lateral shoot formation which also observed to encourage plant growth and improvement under numerous abiotic stress situations (Tuna et al. 2008; Ahmad et al. 2010; Olszewski et al. 2012). SA is well known as a signaling molecule affecting plant growth and development and included in plant immune system responses to pathogens and insect herbivores (Hayat et al. 2010; Bastías et al. 2018). In the present study, *Epichloë* endophyte did not have significant effects on endogenous hormones in most of plants which were inconsistent with the previous researches that *Epichloë* endophyte change hormones to improve host stress tolerance (De Battista et al. 1990; Saikkonen et al. 2004, 2013; Xia et al. 2018). Bunyard and McInnis (1990) reported that E+ tall fescue plants produced significantly more ABA in response to drought stress than did E- plants. Some glasshouse-based research has indicated a similar endophyte-enhancement of ABA concentration in tall fescue leaf tissue in response to drought (Joost 1995). The similar results were also observed in some Chinese native grasses like *Achnatherum inebrians* and *Festuca sinensis*. E+ Chinese wildrye (*Leymus chinensis*) plants have a higher SA content than E- plants, especially when they are exposed to *B. sorokiniana* and *C. lunata* (Wang et al. 2016). Some endophytes have been reported to produce IAA and related indole compounds in culture (De Battista et al., 1990, Yue et al., 2000). The concentration increased in E+ plants may be also adjusted by production of IAA and related compounds *in planta* by endophyte. These phytohormones production *in planta* may also induce the defense related secondary metabolisms in plants. However, far too little is known about the role that hormones may play in the symbiosis and their direct effects on host fitness traits.

In the present study, compare with control, Na^+ ion contents significantly increased (P< 0.05) whereas K^+ ion contents significantly decreased (P< 0.05). Na^+/ K^+ and $S_{K, Na}$ also significantly increased (P< 0.05). Ca^{2+} did not change in most of the plants. Accumulation of inorganic ions such as Na^+ and K^+ is one of mechanism for osmotic adjustment in plants during stress response (Shabala et al. 2011). This is very important for alleviating host damages since Na^+ accumulation in plant cells resulting in extremely damages by inhibiting enzymes, disrupting K^+ acquisition, inhibiting K^+ dependent metabolic processes, and causing oxidative stress (Pan et al. 2016; Zhu 2001). Maintaining constant intracellular K^+ and Na^+ balance is essential for metabolic processes in cells and is crucial for plant adaptation to saline

environments (Zhu 2003). Previous researches have reported that endophyte infection can adjust Na^+ and K^+ concentrations in host plants under stress. For example, Bayat et al. (2009) found that *Epichloë* endophyte increased the K^+ and Ca^{2+} contents in *F. arundinacea* under drought stress. Reza and Mirlohi (2010) showed that *E. coenophiala* and *E. uncinata* endophyte infection reduced Na^+ and Cl^- concentrations in tall fescue and meadow fescue roots but increased K^+ concentrations in the shoots under salt stress. Both Song et al. (2015) and Chen et al. (2018) reported that *E. bromicola* infection reduced Na^+ content, the Na^+/K^+ ratio and shoot Ca^{2+} content but increased K^+ content and the root Ca^{2+} content in *Hordeum brevisubulatum* under salt and alkali stresses. The Na^+ content decline in E+ plant led to plants growing better under stresses compared to E- plants. Restriction of the transport of Na^+ and increase in the K^+ concentration to ensure a high cytosolic K^+/Na^+ ratio are very important for plants to tolerate high salt levels (Berthomieu et al. 2003; Cuin et al. 2003). These changes could decrease the level of toxic ions and osmotic influence on plants under stress treatments. Ca^{2+} is essential for selective ion transport mechanisms and for the maintenance of K^+ influx and Na^+/K^+ selectivity. In our study, *Epichloë* endophyte infection did not show much effects on these indices for most of plants.

In the present study, C and P contents decreased in stress whereas N contents increased. C, N and P are essential elements and the tissue elemental stoichiometry had a mechanistic linkage with the growth rate of the organism. The growth rate hypothesis proposes that higher growth rates are associated with lower C/N, C/P and N/P ratios (Hessen et al. 2007). N availability could stimulate phosphatase activity in the root (Fujita et al. 2010), which could potentially promote P uptake. P is required to meet the protein synthesis demands of increased growth rates (Hessen et al. 2007). *Epichloë* endophyte also adjusted C, N and P contents and C/N and C/P ratios to increased host growth (Song et al. 2015; Song et al. 2016; Chen et al. 2018; Xia et al. 2018). For example, Vázquez-de-Aldana et al. (2013) noted that *E. festucae* alters the nutrient content of *Festuca rubra* regardless of water availability. Song et al. (2015) showed that E+ *H. brevisubulatum* plants had higher contents of N,P and lower ratios of C:N and C:P under salt stress which also confirmed under salt stress and alkali stress by Chen et al. (2018). Xia et al. (2018) reported that E+ *A. inebrians* plants had higher N and P content under soil water deficit. Still, as strong impacts of host genotype, endophyte alleviate these changes only under very few case.

Endophyte-derived alkaloid production is one of the key traits taken into consideration by pasture breeders when selecting endophyte strains for incorporation into a pasture grass breeding program. Alkaloids may play roles help endophyte to confer protection to the plant against abiotic and biotic stress (Nagabhyru et al. 2013; Schardl et al. 2013). For example, alkaloid levels of E+ *Achnatherum inebrians* increased as the NaCl concentration increased, decreased as the water content increased in soil (Zhang et al. 2011). Nagabhyru et al.(2013) showed that loline alkaloid levels increased in response to drought stress in E+ tall fescue. However, the chemical ecology as mediated by endophyte in grasses has been revealed to be far more complex (Saikkonen et al. 2013). The contents variations of N and P contents under stress in our study may also have impacts on alkaloids concentrations as N is an important component for their biosynthesis and P availability influences ergot alkaloid production in endophyte-infected grasses (Belesky et al. 1987; Malinowski et al. 1998; Faeth and Fagan 2002). Alkaloids

production in these individual plants will be evaluated as soon as possible in the near future to provide more comprehensive understanding for the selection and breeding from these materials.

Space mutation results in abundant, non-directional mutations which create genetic variability to improve various complicated traits in plants. Space-induced mutation breading is an effective way to both breed new varieties and enhance genetic diversity (Liu et al. 2008). In the present study, the space mutation had effects on plants performance as there was significant difference (P< 0.05) between mutation and without mutant for all of the tested indices except with plant height, aboveground biomass, CTK contents, underground Na^+ and C/P, $S_{K, Na}$, aboveground C which suggested that the four individuals selected from germplasm with mutation had strong stress tolerance. This is consistent with the primary selection and these individuals had better performance which can be utilized for further breeding. The mutant individuals provided new methods and resources for perennial ryegrass breeding with strong stress tolerance. Using space induced radiation, a number of advantageous mutations to make a breakthrough in most desired crop yield was also achieved. China has produced 41 varieties developed through space–induced mutation breeding of various crop species viz., rice, wheat, cotton, sesame, pepper, tomato, and alfalfa (Liu et al. 2008). However, The space mutation approach results in abundant, non-directional mutations (He et al. 2006). This breeding methodology need followed by lots of work such as material selection, molecular screening of mutants, earlier generation identification of quality characters to successful breed new varieties. We should continue to select excellent individual plants from the second generation of these perennial ryegrass germplasm with space mutation and conduct characterization and genetic analysis in combination with molecular techniques. SSR variation can be used for genetic identification at the level of individuals for each specific host grass-endophyte genotypic association.

There is no report about the effects of space mutation on *Epichloë* endophyte. *Epichloë* endophyte lived inside of E+ seeds which also went through the space induced radiation, possibly, had radiation mutation in genome. The endophyte isolation from E+ plant with mutation and characteristics are in progress to reveal the mutation site and mechanism.

We concluded that endophyte promoted host growth, regulated the rational distribution of ion content and nutrient elements in the host plant. The space mutation also had effects on plants performance. The effects of host genotype on plant performance and stress tolerance was much more pronounced and far greater than that of endophyte infection and mutation. Endophyte had effects on more indices of plants without mutant which suggest mutation may hide some effects of endophyte as its strong effects.

Declarations

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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.

Author contributions

PT designed the experiments, BHM and YL did the experiment and analysis, ZJC, CJL and ZBN provided seeds, BHM, YL, PT, ZJC, CJL and ZBN wrote the manuscript. All authors contributed to the article and approved the submitted version.

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Figures

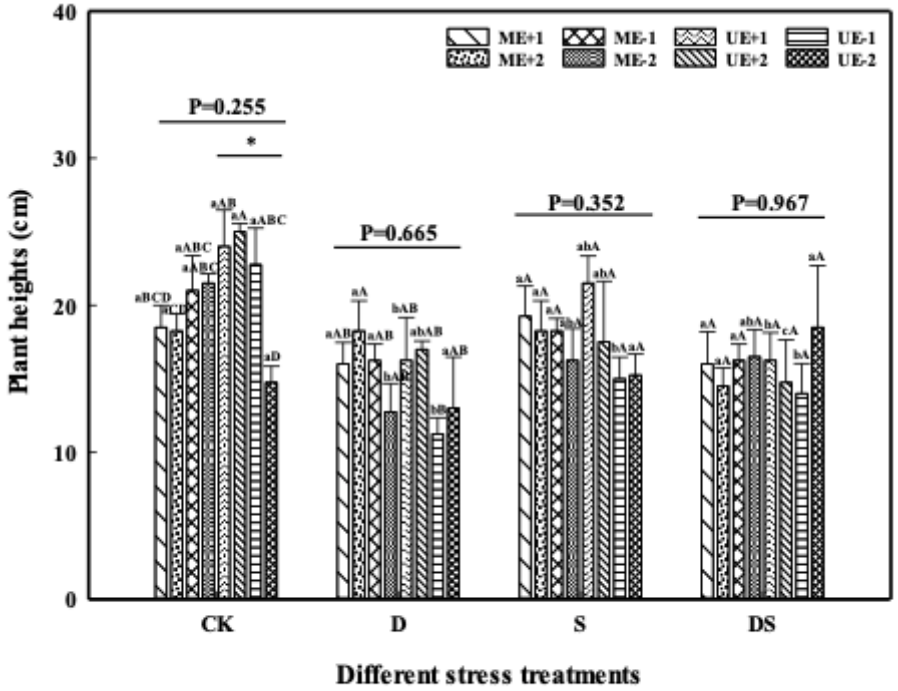

Fig.1

Figure 1

Plant height of different individual plants under different treatments. Note: ME+1 is one genotype from E+ with mutant, ME+2 is another genotypes from E+ with mutant, ME-1 is one genotype from E- with mutant, ME-2 is another genotype from E- with mutant, UE+1 is one genotype from E+ without mutant, UE+2 is another genotypes from E+ without mutant, UE-1 is one genotype from E- without mutant, UE-2 is another genotypes from E- without mutant. CK means control treatment, D means drought stress treatment, S means salt stress treatment and DS means drought with salt stress treatment. Capped lines are standard errors of the mean. Lower case letters compare the same plants under different treatments, upper case letters compare the different plants under the same treatment, * compare the difference between E+ plants and E- plants within the mutation or without mutation under the same treatment, P value compare the difference between plants with mutation and plants without mutation under the same treatment.

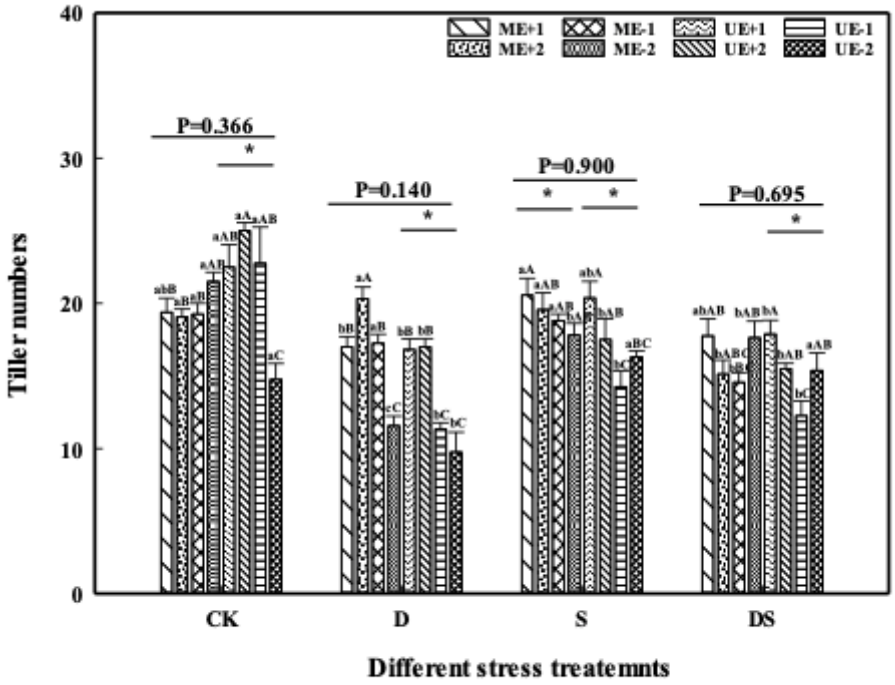

Fig.2

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Figure 2

Tiller number of different individual plants under different treatments. Note: the same as Fig.1.

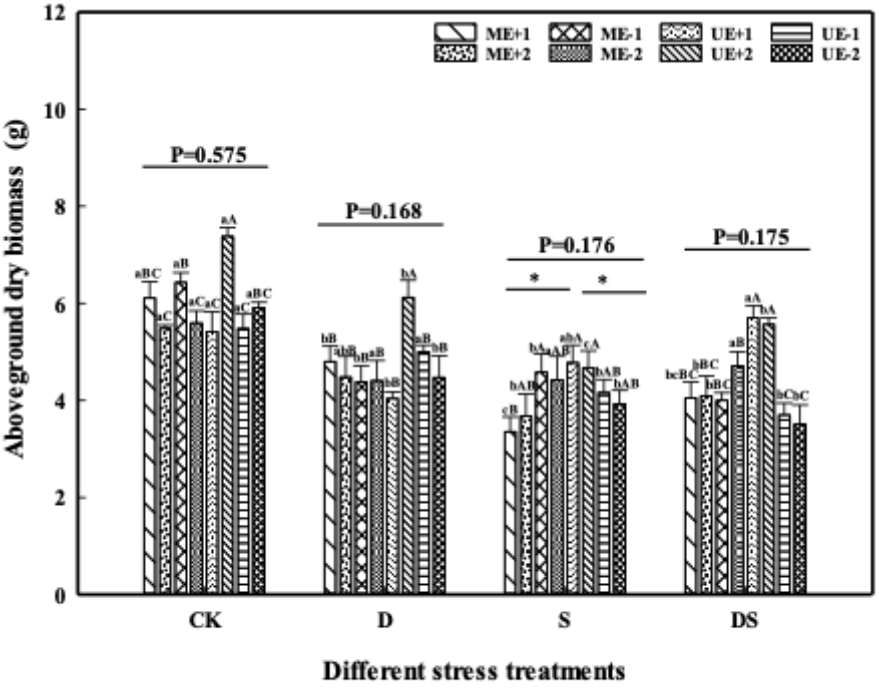

Fig.3

Figure 3

Aboveground dry biomass of different individual plants under different treatments. Note: the same as Fig.1.

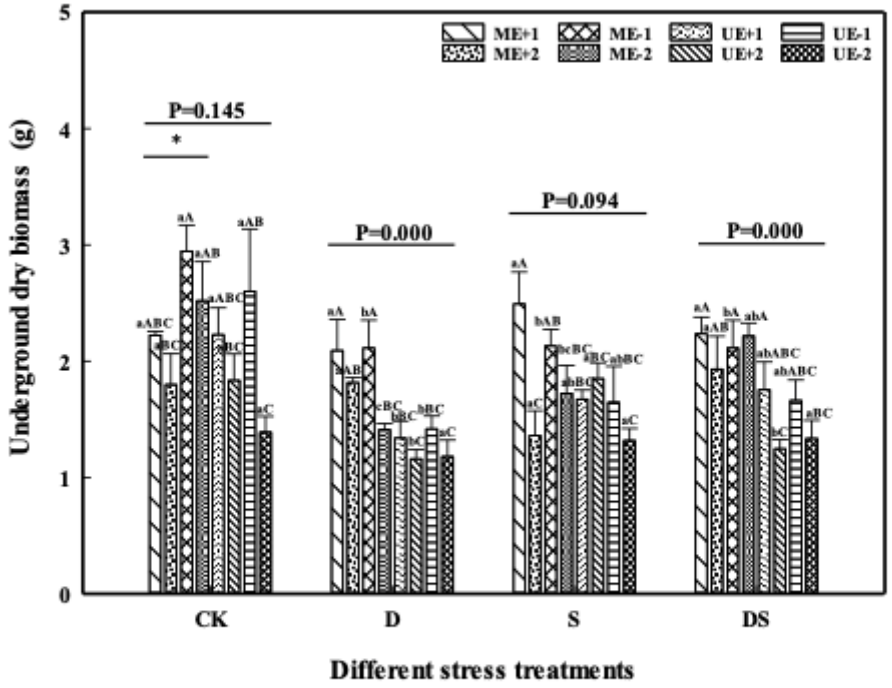

Fig.4

Figure 4

Underground dry biomass of different individual plants under different treatments. Note: the same as Fig.1.

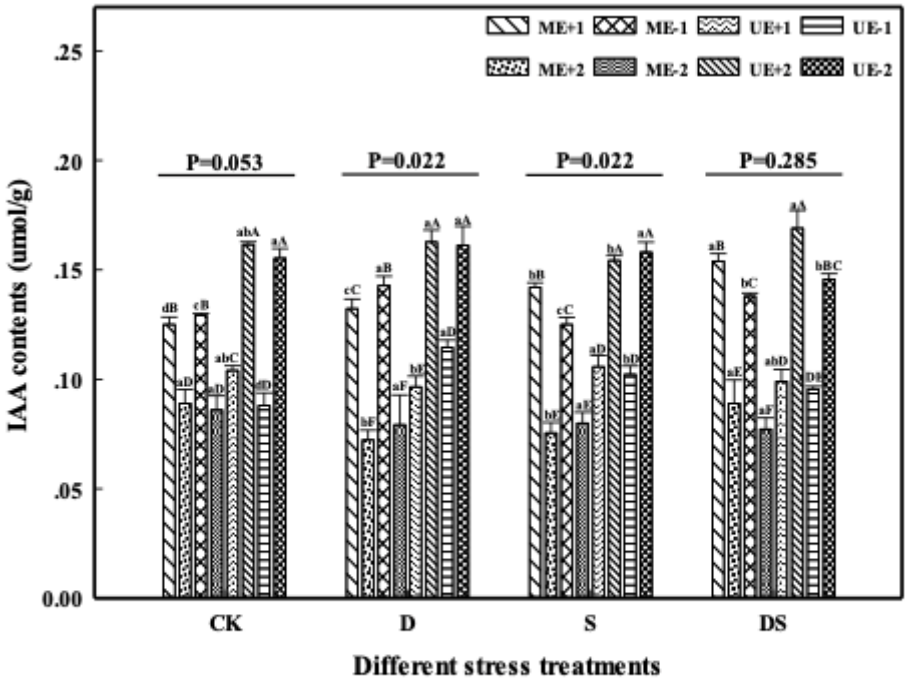

Fig.5

Figure 5

IAA contents of different individual plants under different treatments. Note: the same as Fig.1

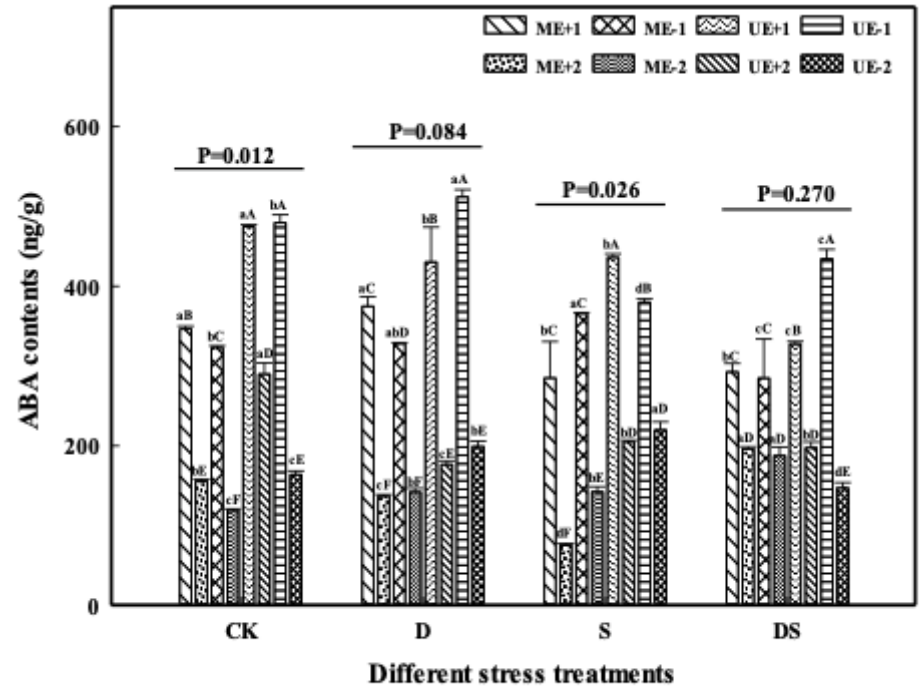

Fig.6

Figure 6

ABA contents of different individual plants under different treatments. Note: the same as Fig.1

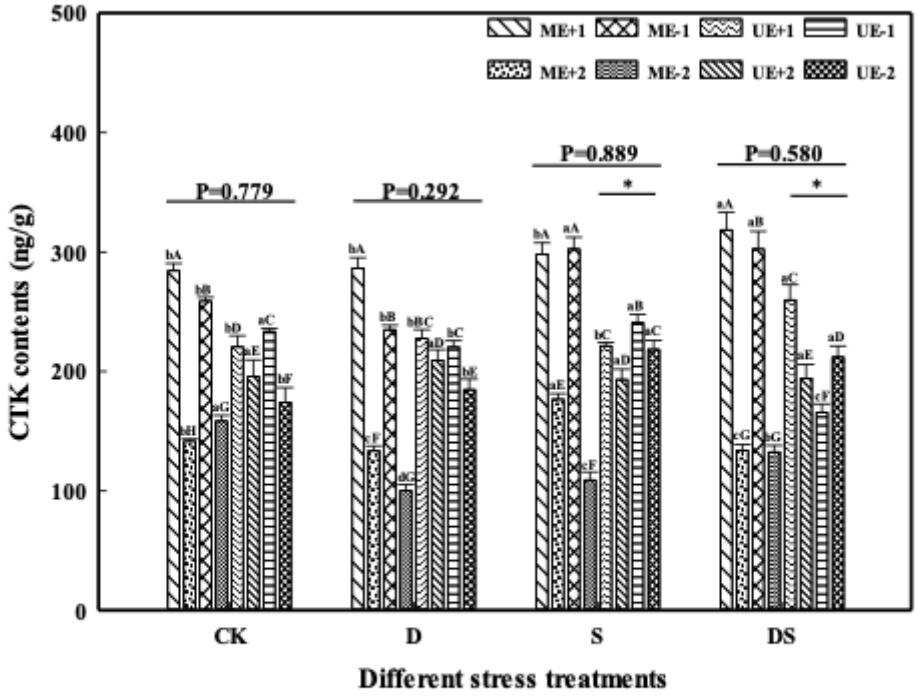

Fig.7

Figure 7

CTK contents of different individual plants under different treatments. Note: the same as Fig.1

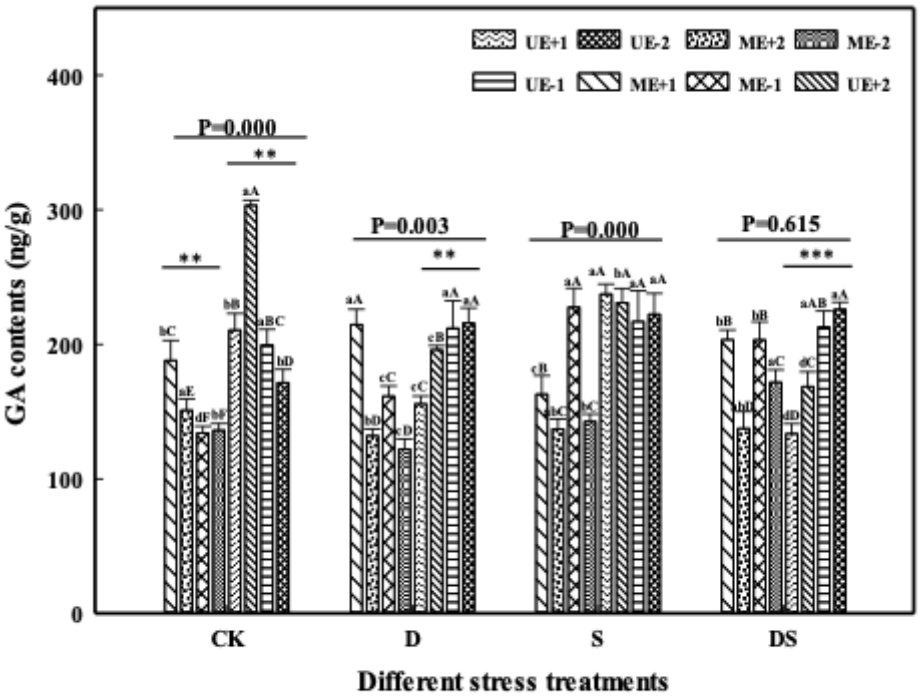

Fig.8

Figure 8

GA contents of different individual plants under different treatments. Note: the same as Fig.1

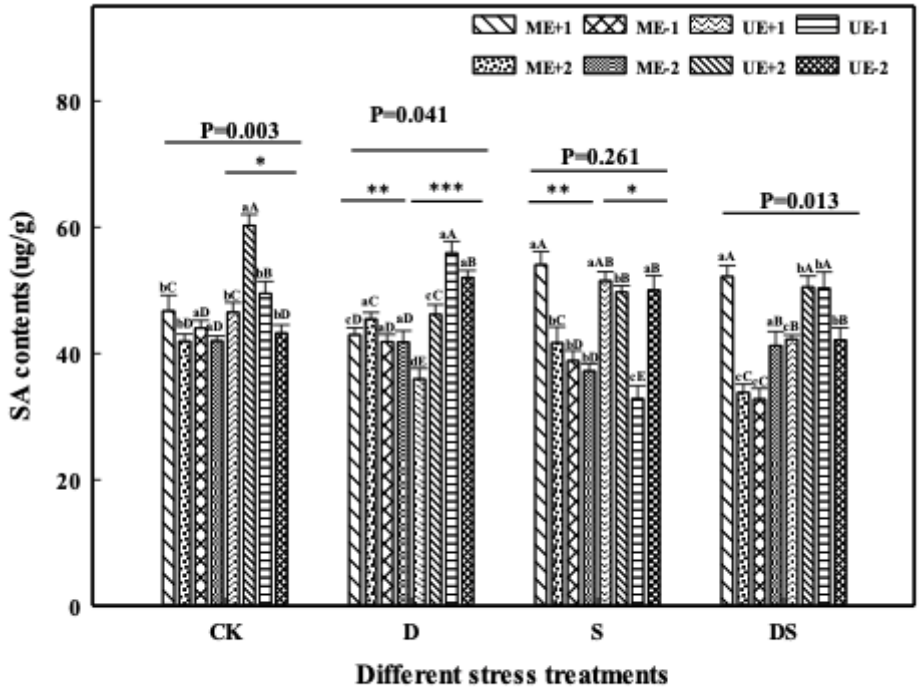

Fig.9

Figure 9

SA contents of different individual plants under different treatments. Note: the same as Fig.1

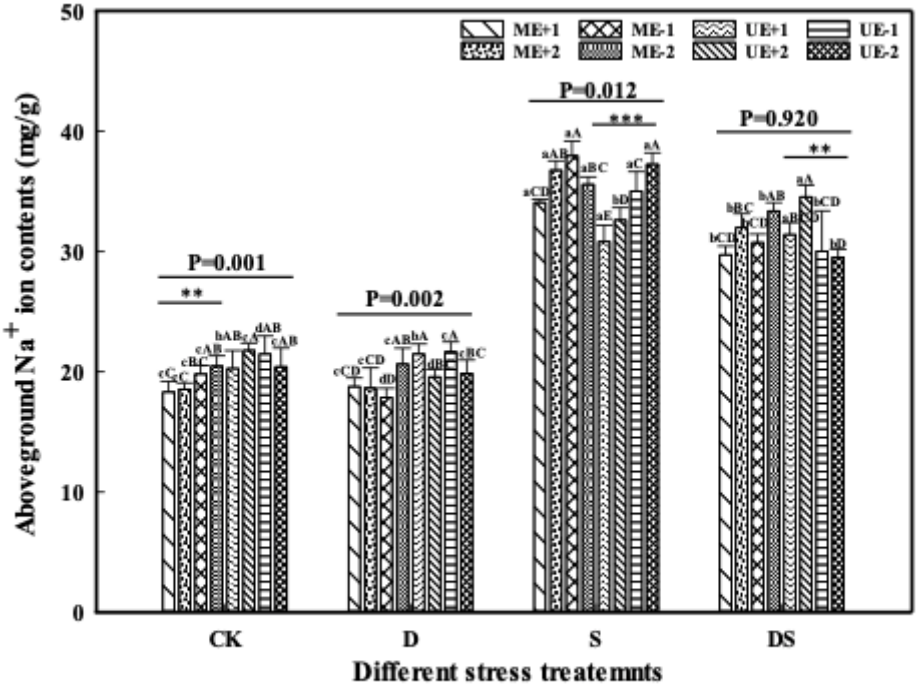

Fig.10

Figure 10

Aboveground Na+ ion contents of different individual plants under different treatments. Note: the same as Fig.1

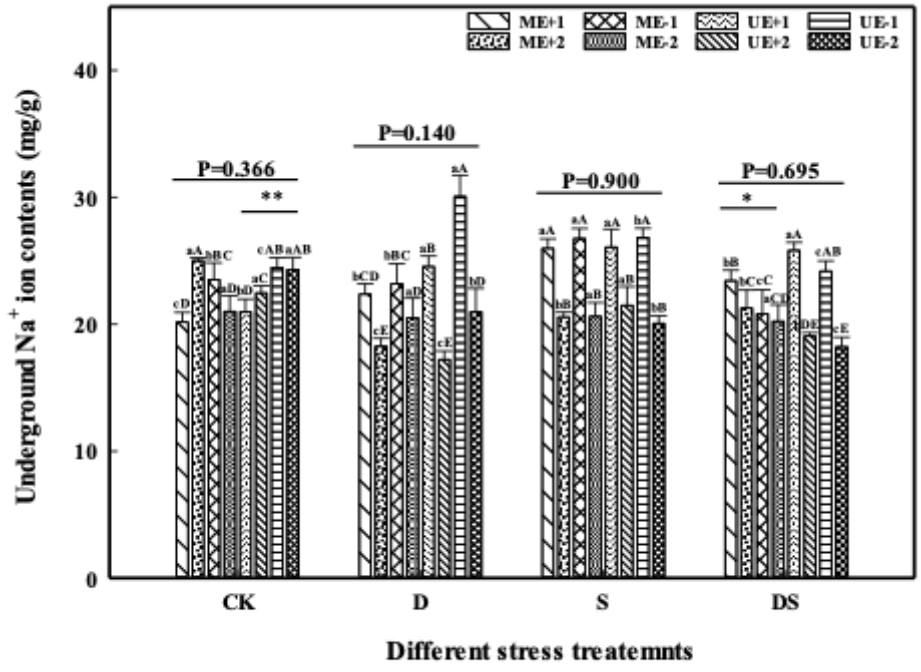

Fig.11

Figure 11

Underground Na+ ion contents of different individual plants under different treatments. Note: the same as Fig.1

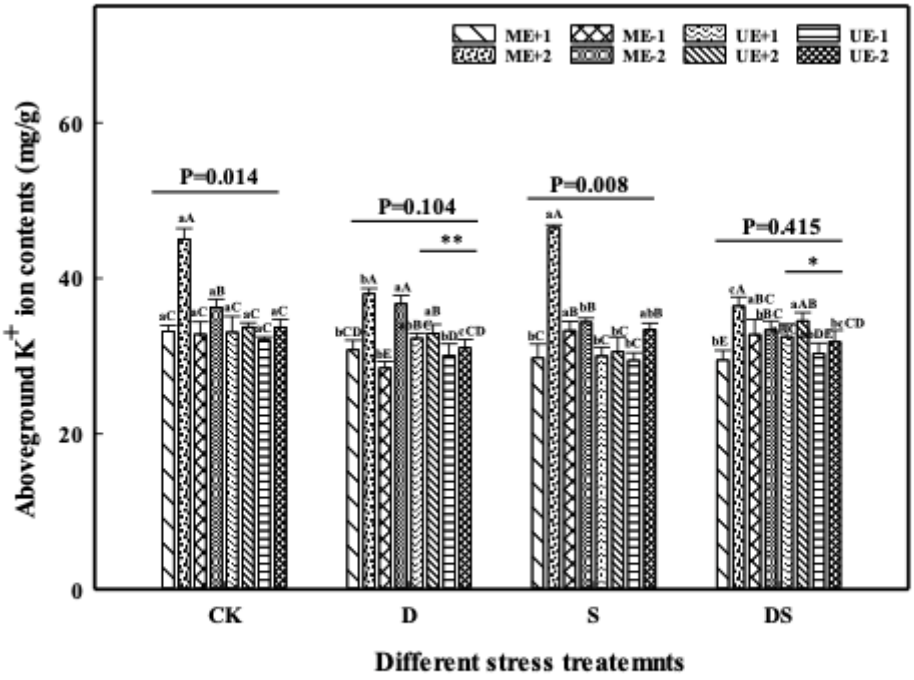

Fig.12

Figure 12

Aboveground K+ ion contents of different individual plants under different treatments. Note: the same as Fig.1

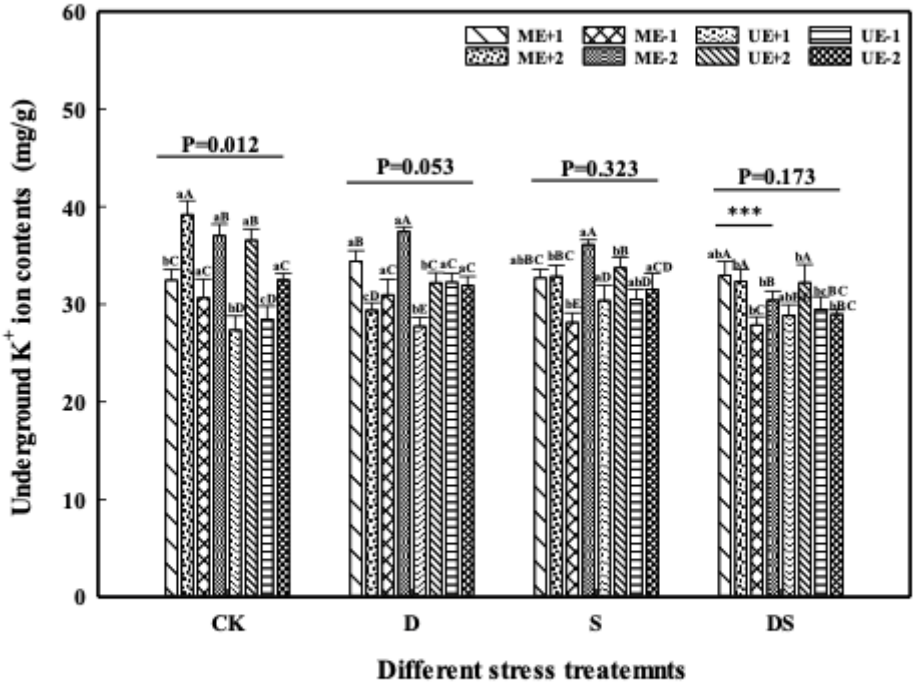

Fig.13

Figure 13

Underground K+ ion contents of different individual plants under different treatments. Note: the same as Fig.1

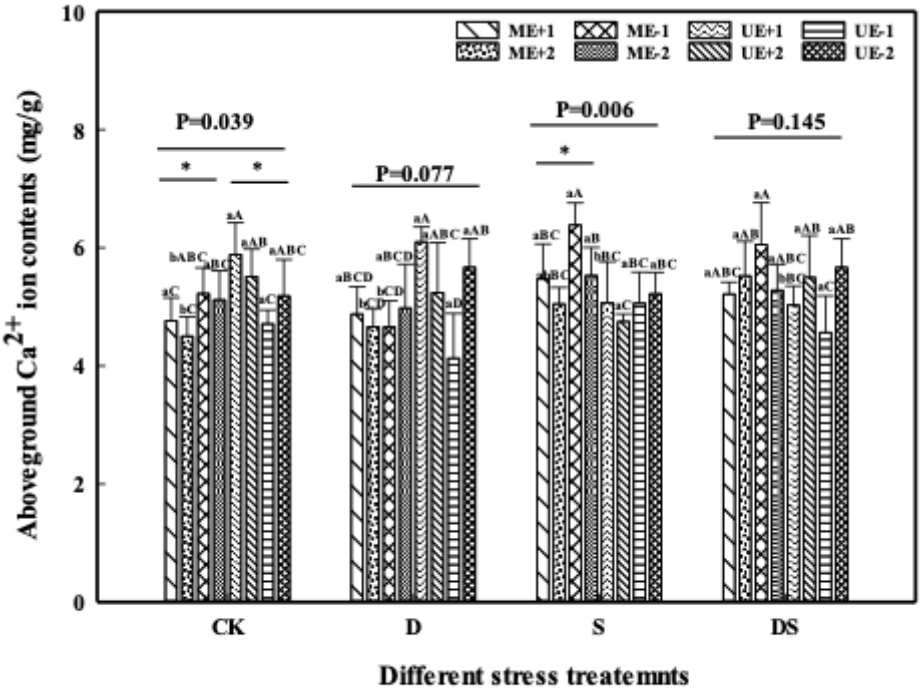

Fig.14

Figure 14

Aboveground Ca2+ ion contents of different individual plants under different treatments. Note: the same as Fig.1

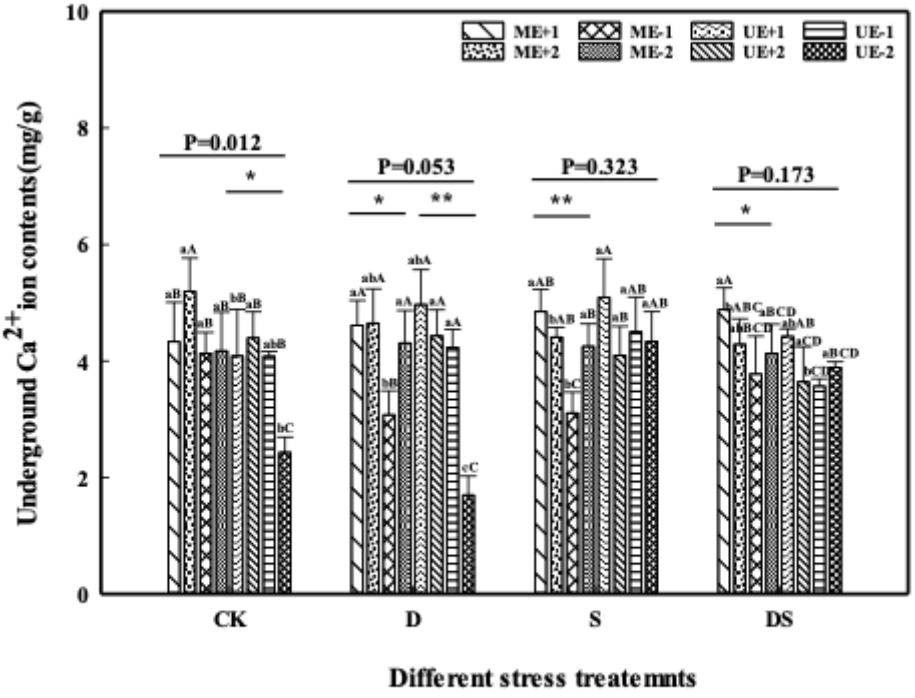

Fig.15

Figure 15

Underground Ca2+ion contents of different individual plants under different treatments. Note: the same as Fig.1

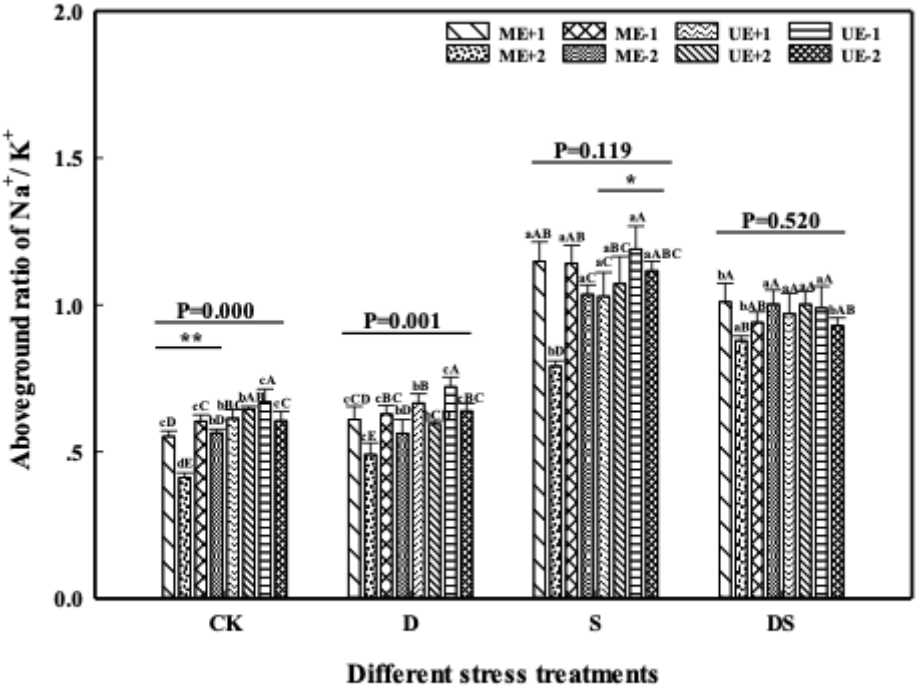

Fig.16

Figure 16

Aboveground ratio of Na+/ K+ of different individual plants under different treatments. Note: the same as Fig.1

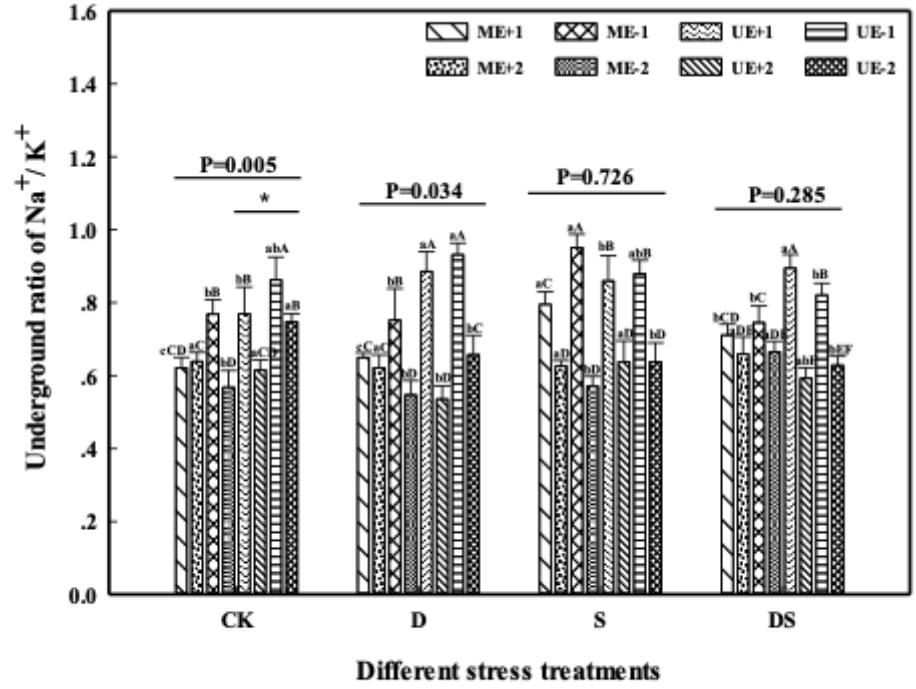

Fig.17

Figure 17

Underground ratio of Na+/ K+ of different individual plants under different treatments. Note: the same as Fig.1

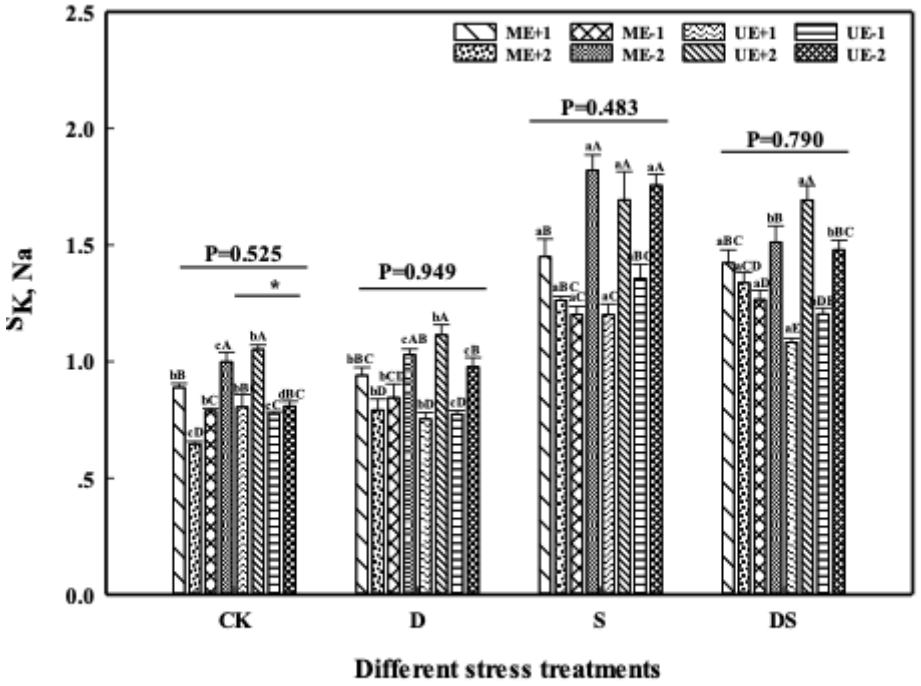

Fig.18

Figure 18

SK, Na of different individual plants under different treatments. Note: the same as Fig.1

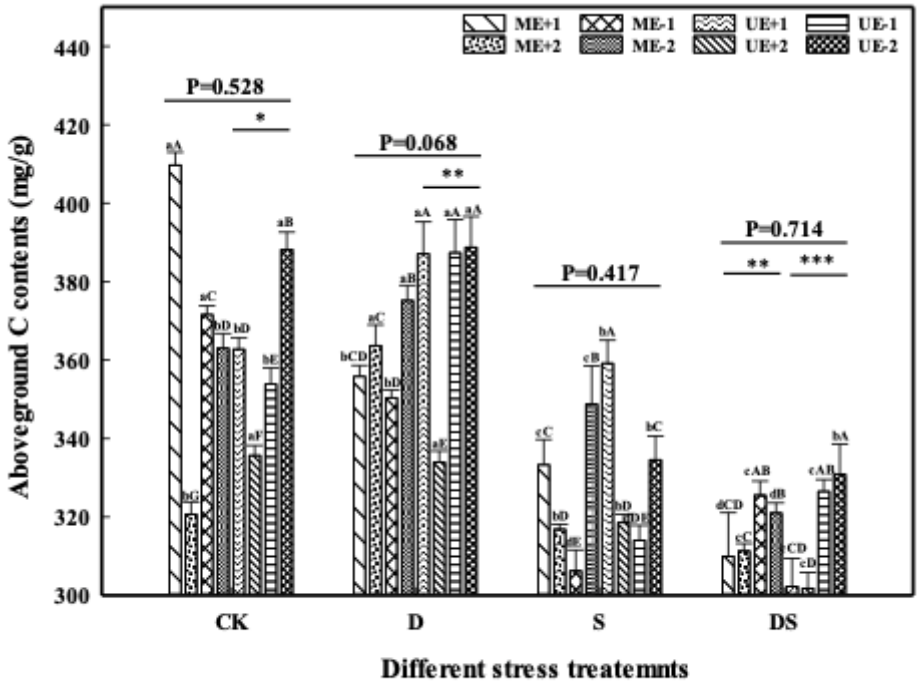

Fig.19

Figure 19

Aboveground C contents of different individual plants under different treatments. Note: the same as Fig.1

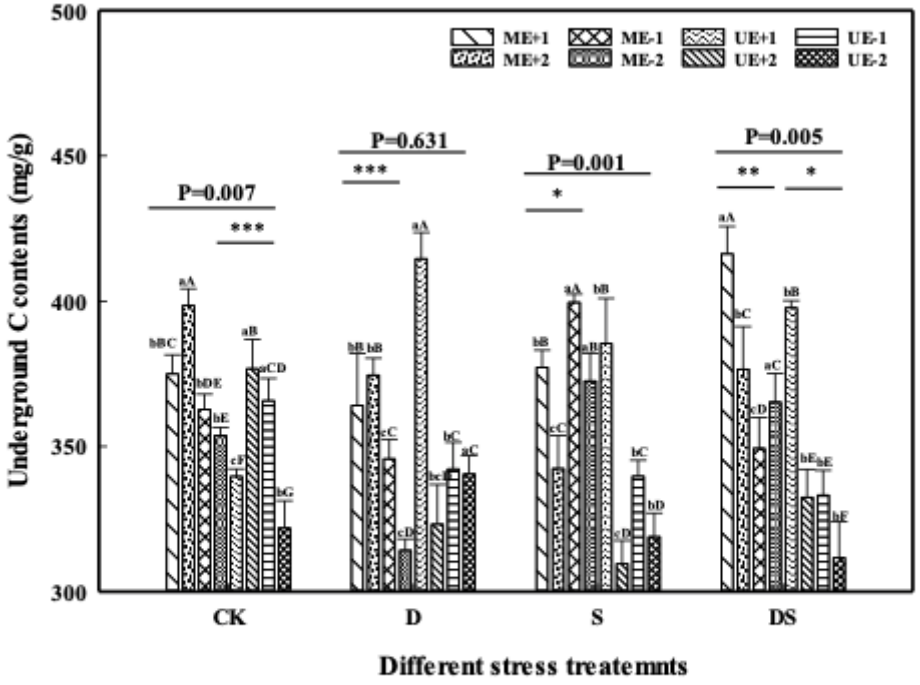

Fig.20

Figure 20

Underground C contents of different individual plants under different treatments. Note: the same as Fig.1

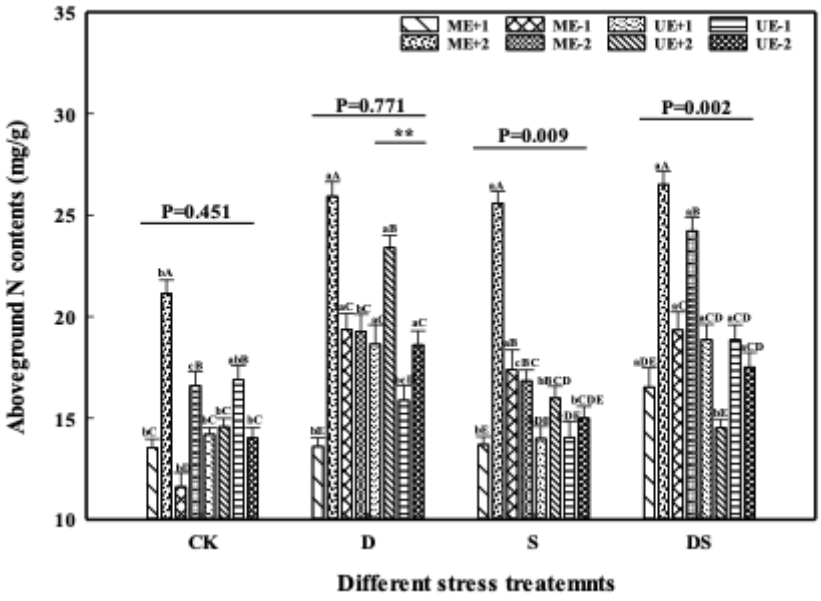

Figure 21

aboveground N contents of different individual plants under different treatments. Note: the same as Fig.1

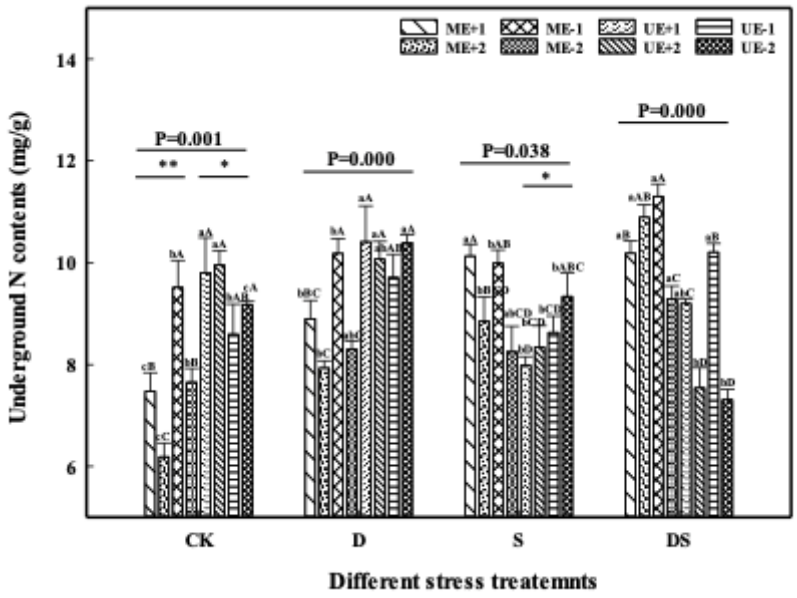

Figure 22

Underground N contents of different individual plants under different treatments. Note: the same as Fig.1

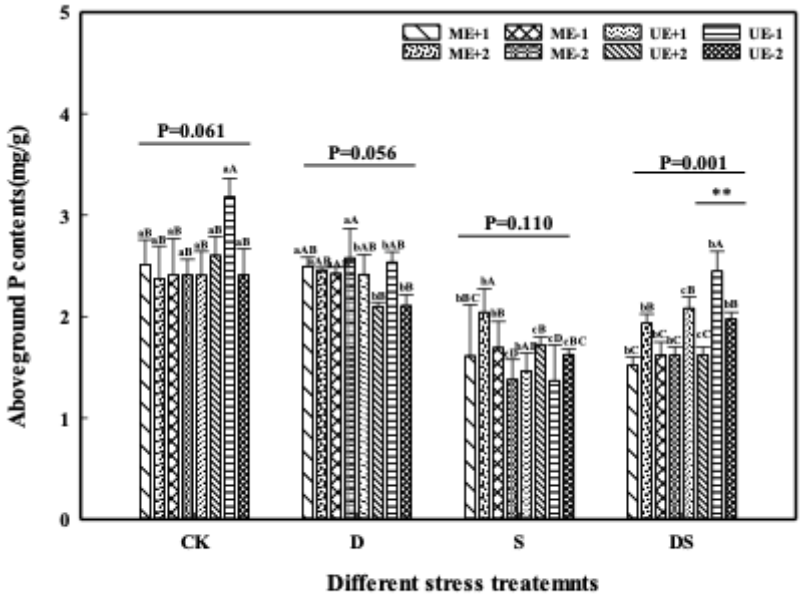

Fig.23

Figure 23

Aboveground P contents of different individual plants under different treatments. Note: the same as Fig.1

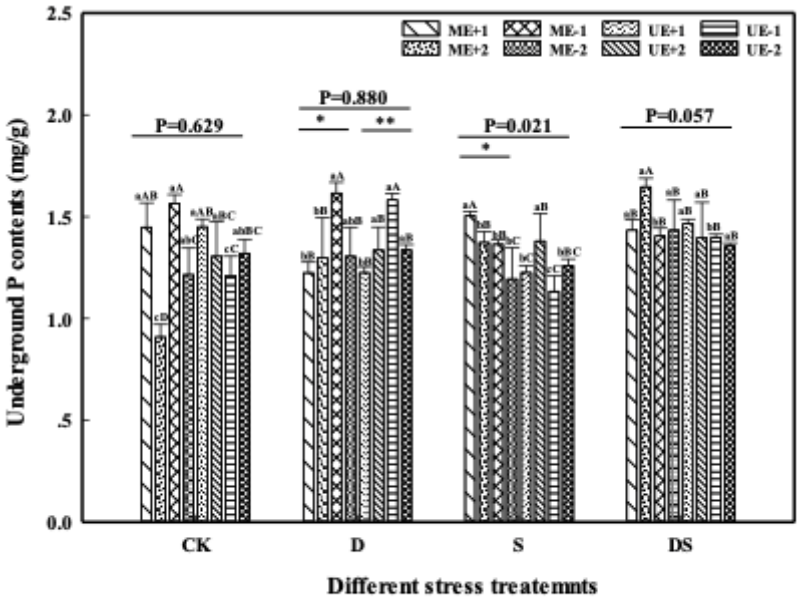

Fig.24

Figure 24

Underground P contents of different individual plants under different treatments. Note: the same as Fig.1

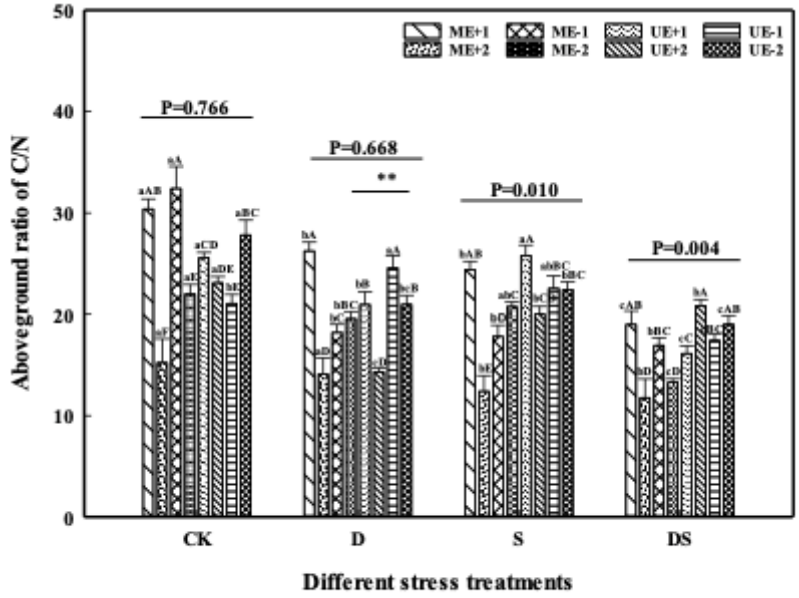

Fig.25

Figure 25

Aboveground ratio of C/N of different individual plants under different treatments. Note: the same as Fig.1

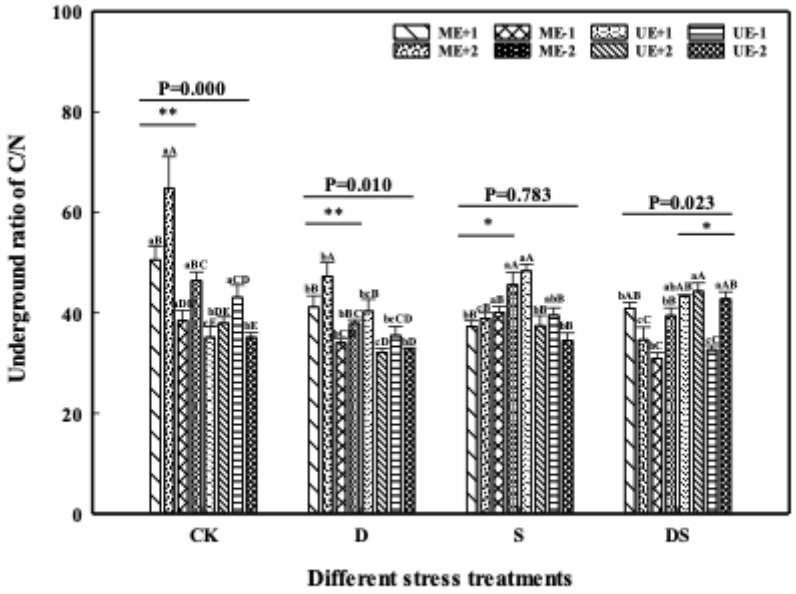

Fig.26

Figure 26

Underground ratio of C/N of different individual plants under different treatments. Note: the same as Fig.1

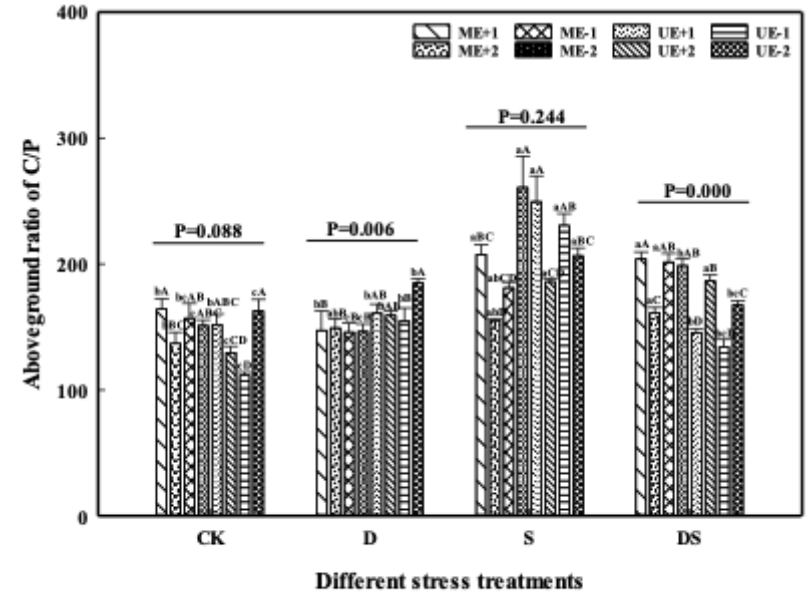

Fig.27

Figure 27

Aboveground ratio of C/P of different individual plants under different treatments. Note: the same as Fig.1

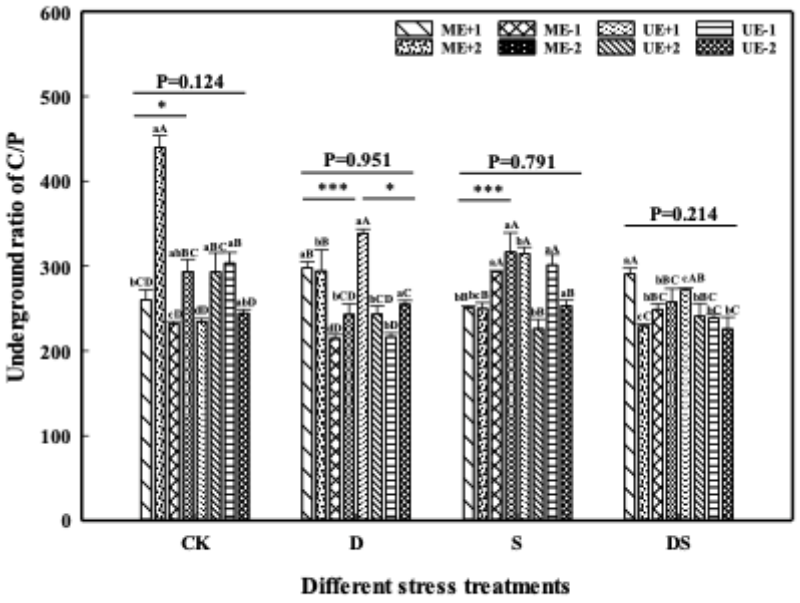

Fig.28

Figure 28

Underground ratio of C/P of different individual plants under different treatments. Note: the same as Fig.1

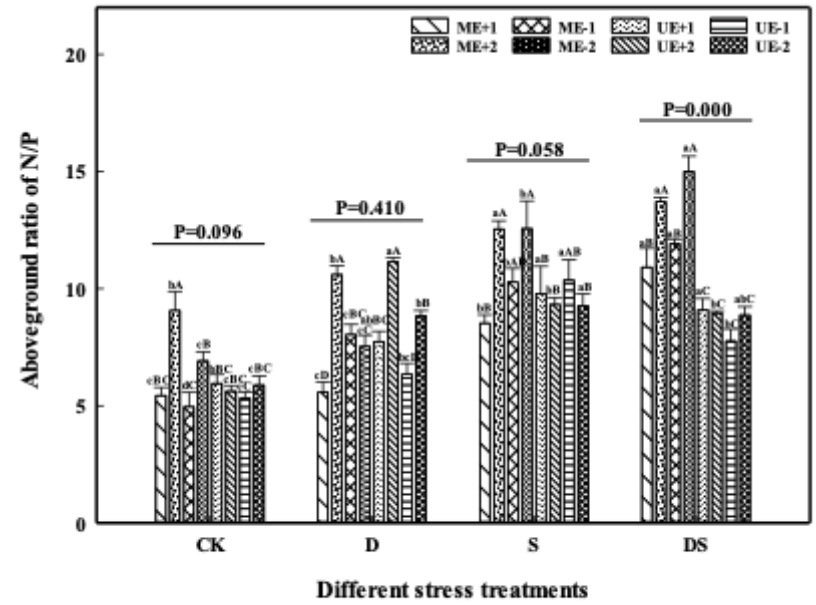

Fig.29

Figure 29

Aboveground ratio of N/P of different individual plants under different treatments. Note: the same as Fig.1

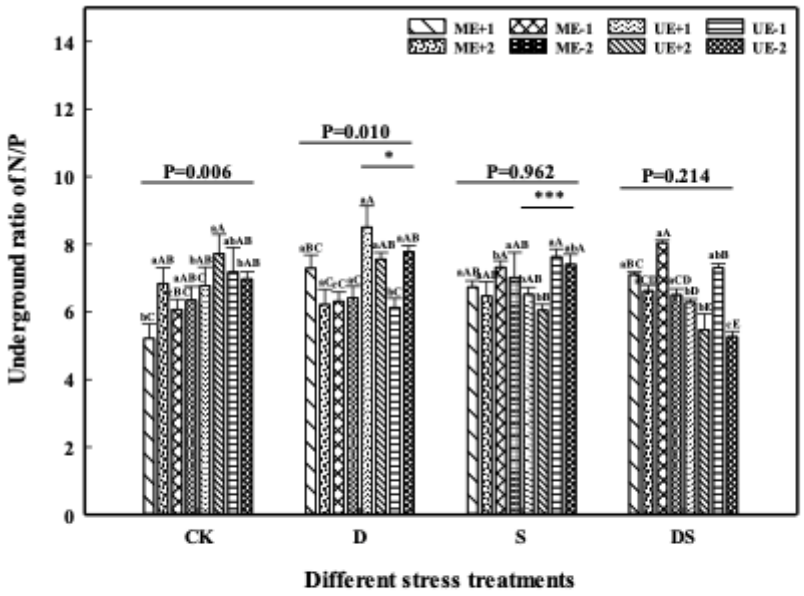

Fig.30

Figure 30

Underground ratio of N/P of different individual plants under different treatments. Note: the same as Fig.1

Supplementary Files

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- TableS1.xlsx