Effects of Mixed Saline-Alkali Stress on the Growth of Ground-Cover Chrysanthemum 'Hanlu Hong' Postprint

Authors: Dang Peipei, Li Mingyu, Zhao Zhe, Wang Ruoshui, Cheng Jin, Huijie Xiao

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Abstract

Saline-alkali stress, as one of the major abiotic stresses, has become a critical constraint on crop production and ecological environment construction in China. The Qingtongxia region of Ningxia is a Yellow River irrigation area where soil salinization has led to landscaping being dominated by halophytes, with a scarcity of flowering species. This study employed ground-cover chrysanthemum 'Hanluhong'(Chrysanthemum morifolium 'Hanluhong') as experimental material to investigate, on one hand, the effects of mixed saline-alkali stress on its growth, and on the other hand, its adaptability in the Qingtongxia region by simulating local saline-alkali stress levels. The experiment established three pH gradients (7.0, 8.0, 9.0), and at each pH gradient, prepared mixed solutions of varying concentrations (0, 0.2%, 0.4%, 0.6%, 0.8%, 1.0%) using NaCl, Na2CO3, NaHCO3, and Na2SO4 to treat ground-cover chrysanthemum plants, observing and measuring changes in plant height, root length, photosynthetic characteristics, and chlorophyll fluorescence parameters under different stress conditions. The experimental results demonstrated: (1) Prolonged growth in high saline-alkali environments resulted in retarded growth of ground-cover chrysanthemum 'Hanluhong', with concomitant declines in photosynthetic parameters and chlorophyll fluorescence parameters. (2) In the Qingtongxia experimental simulation group (pH=8.0, salt concentration of 0.4%), the elongation rates of both plant height and root length exhibited a trend of initial decrease followed by increase with extended stress duration; simultaneously, while photosynthetic parameters and chlorophyll fluorescence parameters showed declining trends, the reduction in fluorescence parameters did not attain statistical significance. Comprehensive analysis revealed that both high-salt and high-alkali environments are detrimental to the growth and development of ground-cover chrysanthemum 'Hanluhong' plants. Under the saline-alkali stress intensity characteristic of the Qingtongxia region, ground-cover chrysanthemum 'Hanluhong' possesses certain saline-alkali

tolerance, can essentially maintain normal growth, and may be utilized for local vegetation restoration, ecological environment restoration, and landscape design.

Full Text

Effects of Saline-Alkali Stress on the Growth of Chrysanthemum morifolium ‘Hanluhong’

DANG Peipei[1], LI Mingyu[1], ZHAO Zhe[1], WANG Ruoshui[2], CHENG Jin[1], XIAO Huijie[2]*

[1]College of Biological Sciences and Technology, Beijing Forestry University, Beijing 100083, China
[2]School of Soil and Water Conservation, Beijing Forestry University, Beijing 100083, China

Abstract

Saline-alkali stress, as one of the major abiotic stresses, has become a severe constraint on crop production and ecological environment construction in China. The Qingtongxia area of Ningxia belongs to the Yellow River irrigation region, where soil salinization has led to landscaping dominated by halophytes with a scarcity of ornamental flowers. This study used ground-cover chrysanthemum ‘Hanluhong’ (Chrysanthemum morifolium ‘Hanluhong’) as experimental material to investigate the effects of mixed saline-alkali stress on its growth and to evaluate its adaptability in the Qingtongxia region by simulating local stress conditions. The experiment established three pH gradients (7.0, 8.0, 9.0) and prepared mixed solutions with different concentrations (0, 0.2%, 0.4%, 0.6%, 0.8%, 1.0%) using NaCl, Na CO , NaHCO , and Na SO at each pH level to treat chrysanthemum plants. Plant height, root length, photosynthetic characteristics, and chlorophyll fluorescence parameters were measured under different stress conditions. Results showed that: (1) prolonged growth in high saline-alkali environments inhibited growth and decreased photosynthetic and chlorophyll fluorescence parameters in ‘Hanluhong’ ; (2) in the Qingtongxia simulation group (pH=8.0, salt concentration 0.4%), the elongation rates of plant height and root length initially decreased then increased with extended stress duration; meanwhile, photosynthetic and chlorophyll fluorescence parameters showed declining trends, though fluorescence parameter reductions were not statistically significant. Comprehensive analysis indicates that both high-salt and high-alkali environments are detrimental to the growth and development of ‘Hanluhong’. However, under the saline-alkali stress intensity of the Qingtongxia region, ‘Hanluhong’ exhibits certain saline-alkali tolerance and can basically grow normally, making it suitable for local vegetation restoration, ecological remediation, and landscape design.

Keywords: mixed saline-alkali stress, Chrysanthemum morifolium Ramat., plant height, root length, photosynthetic characteristics, chlorophyll fluorescence

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Saline-alkali stress represents one of the primary abiotic factors limiting plant growth and development, severely impacting agricultural production, ecological environments, and sustainable development in affected regions (Wang et al., 2017). Ground-cover chrysanthemum (Chrysanthemum morifolium Ramat.) is a perennial herbaceous plant in the Asteraceae family developed through long-term artificial hybridization, characterized by dense flowering, diverse colors, and extended blooming periods, offering high ornamental value (Liao et al., 2010). Additionally, ground-cover chrysanthemum demonstrates strong adaptability, saline-alkali tolerance, and drought resistance, making it an important landscaping plant with significant applications in scenery design, environmental beautification, and ecological restoration. Previous studies have shown that ground-cover chrysanthemum can adapt well to cold, high-temperature, and drought conditions, indicating high application value (Cui, 2005; Jing et al., 2015; Zhang et al., 2016). In recent years, increasing attention has focused on its saline-alkali resistance characteristics, with research by Shi et al. (2010) and Zhang (2016) demonstrating that ground-cover chrysanthemum maintains relatively stable physiological indicators under certain salt concentrations, establishing it as a ground-cover flower with strong salt resistance. In studies introducing ground-cover plants to saline-alkali regions, ground-cover chrysanthemum has been promoted as a premium species for saline-land improvement due to its good adaptability and stress resistance (Zhang et al., 2010).

Salt stress affects morphological indicators including seedling height, root length, root number, and biomass accumulation (Wang et al., 2010; Liu et al., 2014), while high salt concentrations disrupt ionic and osmotic balance within plants (Ruiz et al., 2016). Alkali stress encompasses the same stress factors as salt stress, but high pH environments additionally impair root growth and development, hindering mineral element absorption and ionic homeostasis reconstruction (Guo et al., 2016). When coping with alkali stress, plants must not only regulate intracellular pH but also consume substantial materials and energy to adjust rhizosphere microenvironment pH, making its damage significantly greater than salt stress alone (Shi & Wang, 2005). In China, most saline-alkali regions contain mixed saline-alkali soils with multiple coexisting salts, which exert far greater impacts on plants than single salt or alkali stress. Regarding photosynthesis, ionic and osmotic stress from salt solutions cause water loss, reducing stomatal conductance and transpiration rate (Li et al., 2015), while alkaline environments readily precipitate Mg^2 , hindering chlorophyll synthesis (Lu et al., 2007). Mixed saline-alkali stress not only causes these damages but also induces significant structural changes in chloroplasts under severe stress, damaging photosystem reaction centers and inhibiting photosynthetic electron transport and photosystem activity (Kalaji et al., 2016). Research by Ju (2008) and Liu et al. (2015) on oat seedlings confirmed that both salt stress and mixed saline-alkali stress inhibit oat seedling growth, causing photosynthetic and chlorophyll fluorescence parameters to decrease with increasing salt concentration, with more pronounced reductions under mixed saline-alkali stress. Beyond affecting photosynthetic systems, mixed saline-alkali stress alters plant structure, including thinner leaves with relatively increased palisade tissue proportion, smaller and thinner stem epidermal cells with thickened cuticles, reduced vessel numbers, and impaired pollen morphology and stigma receptivity under prolonged stress, consequently affecting plant growth and development (Wang, 2010; Niu, 2013; Tian et al., 2014).

China' s severely salinized regions in the northwest, northeast, north China, and coastal areas predominantly feature mixed saline-alkali soils with multiple salts; therefore, mixed saline-alkali stress simulation experiments more accurately reflect plant saline-alkali tolerance. The ability of ground-cover chrysanthemum to maintain good growth status under mixed saline-alkali stress constitutes an important prerequisite for its promotion in saline-alkali regions. Among the 441,100 hectares of cultivated land in Ningxia' s Yellow River irrigation region, 147,900 hectares are salinized. Qingtongxia area, located in the upper Yellow River and central Ningxia Plain, has extensive saline-alkali cultivated land accounting for 89% of the total salinized cultivated area in the irrigation region (Huang, 2010). Research by Fan et al. (2012) indicates that soil anions and cations in Ningxia primarily include Cl , CO ^{2} , HCO , SO ^{2} , Na , K , Ca^{2} , and Mg^{2} , constituting a multi-salt mixed saline-alkali soil. Saline-alkali concentrations vary across different locations in Qingtongxia, but generally, the 0-80 cm soil layer has total salt concentrations of 0.384-0.409% with saturated paste extract pH around 8.0. Dominant vegetation includes Tamarix chinensis, Suaeda salsa, and Phragmites australis (Yang et al., 2018), indicating a state dominated by halophytes with scarce ornamental flowers. This study selected the salt-resistant ground-cover chrysanthemum cultivar 'Hanluhong' (Chrysanthemum morifolium 'Hanluhong') to investigate mixed saline-alkali stress effects on its growth while simulating Ningxia Qingtongxia soil conditions. By measuring morphological data, photosynthetic characteristics, and chlorophyll fluorescence parameters, we assessed ' Hanluhong' s growth status under Qingtongxia saline-alkali stress levels to evaluate its adaptability in Ningxia' s saline-alkali soils, providing theoretical and practical foundations for species selection in local vegetation restoration and landscaping.

1.1 Materials

Ground-cover chrysanthemum 'Hanluhong' plants were provided by the National Engineering Research Center for Floriculture. Cuttings were propagated in a greenhouse on June 11, 2017, and transplanted on July 16 into a substrate mixture of peat:vermiculite:perlite (2:1:1 v:v:v). After conventional management and seven days of stabilization, 360 uniform, healthy plants were selected for the experiment. During the trial, soil moisture was maintained by timed sprinkler irrigation for one minute each afternoon.

1.2.1 Mixed Saline-Alkali Stress Treatment

Based on Li et al. (2015) classification standards for mild saline-alkali (soil salt content <0.2% in 1 m soil layer), moderate saline-alkali (0.2%-0.6%), and severe saline-alkali (0.6%-1.0%), experimental solutions were prepared using NaCl, Na CO , NaHCO , and Na SO at a 1:1:1:1 mass ratio to create mixed saline-alkali solutions at concentrations of 0, 0.2%, 0.4%, 0.6%, 0.8%, and 1.0%, with pH gradients of 7.0, 8.0, and 9.0. The treatment with pH=8.0 and 0.4% concentration most closely approximated Qingtongxia soil conditions. Considering factor interactions, the experiment comprised 18 treatment groups: pH=7.0, 8.0, and 9.0 groups were designated as A, B, and C respectively, with salt concentrations from low to high designated as 0, 1, 2, 3, 4, and 5 (e.g., pH=7.0 with 0% concentration was A0). Each treatment group contained 20 plants, receiving 100 mL of saline-alkali solution every five days for three applications. Plant height, root length, photosynthetic characteristics, and chlorophyll fluorescence parameters were measured two days after each treatment.

1.2.3 Photosynthetic Characteristic Parameters Measurement

Photosynthetic parameters were measured outdoors between 9:00-10:00 AM using a portable photosynthesis system Li-6400 (LI-COR, USA) with natural light leaf chambers. The fourth to fifth fully expanded leaves from the top were selected for measurement of net photosynthetic rate (Pn, mol CO ·m ^{2}·s ^{1}), transpiration rate (Tr, mmol H O·m ^{2}·s ^{1}), stomatal conductance (Gs, mmol H O·m ^{2}·s ^{1}), and intercellular CO concentration (Ci, mol CO ·mol ^{1}). Three plants were measured per treatment with six replicate readings per plant.

1.2.4 Chlorophyll Fluorescence Parameters Measurement

Chlorophyll fluorescence parameters were measured using a pulse-amplitude-modulation fluorometer PAM-2500 (WALZ, Germany). Initial fluorescence (Fo) and maximum fluorescence (Fm) were recorded, and Fv/Fm = (Fm-Fo)/Fm (maximum photochemical efficiency of PSII) and Fv/Fo = (Fm-Fo)/Fo (potential photochemical activity of PSII) were calculated. Instrument settings were: measuring light intensity 860 mol·m ^{2}·s ^{1}, actinic light intensity 1300 mol·m ^{2}·s ^{1}, saturation pulse intensity 3450 mol·m ^{2}·s ^{1}, and pulse duration 0.5 s. All measurements were preceded by 25 minutes of dark adaptation.

1.3 Data Processing and Statistical Analysis

Data were processed using Microsoft Office Excel 2016. Significance analysis of plant height, root length, photosynthetic parameters, and chlorophyll fluorescence parameters under different mixed saline-alkali stresses was performed using SPSS 20.0. SigmaPlot 12.5 was used for figure preparation.

2.1 Effects of Different Saline-Alkali Stress Treatments on Plant Height

Pre-stress average plant height was 12.42 ± 0.81 cm (n=6). As shown in Table 1 , plant height increased in both A and B treatment groups after 15 days of stress. Group A showed increases of 22.11%, 25.60%, 34.46%, 29.90%, 33.92%, and 9.23%, while Group B increased by 28.82%, 25.07%, 22.65%, 40.10%, 31.78%, and 18.89%. In both A and B groups, plant height initially increased then decreased with rising salt concentrations, with significant changes (F=3.269, $P<0.05$; F=4.118, $P<0.05$). The A5 and B5 treatments showed the smallest growth increments after 15 days. In Group C, plant height increased after 5 and 10 days of stress, but decreased after 15 days compared to day 10 in all treatments except C0. The B2 treatment, which most closely simulated Qingtongxia conditions, showed a 9.50% increase in plant height after 5 days of stress, minimal growth (0.14%) between days 5-10, and resumed growth with a 9.56% increase between days 10-15.

2.2 Effects of Different Saline-Alkali Stress Treatments on Root Length

Root length directly reflects root growth status as roots are the primary organs affected by saline-alkali stress. Pre-stress root length was 12.63 ± 0.76 cm (n=6). As shown in Table 2 , after 15 days of stress, Group A root lengths increased by 31.70%, 33.28%, 26.95%, 16.65%, 25.10%, and 23.78%; Group B increased by 34.34%, 31.96%, 32.49%, 28.00%, 27.47%, and 28.53%; while Group C, most severely affected, showed the smallest increments of 20.61%, 18.24%, 15.60%, 15.60%, 10.58%, and 7.42%. After 15 days, all Group A treatments except A4 showed significant root elongation, while in Group B only B0 and B1 were significant, and Group C showed no significant changes. The Qingtongxia simulation group (B2) exhibited 16.39% root elongation after 5 days, slow growth (1.15%) between days 5-10, and accelerated growth (12.52%) between days 10-15.

2.3 Effects of Different Saline-Alkali Stress Treatments on Net Photosynthetic Rate

Changes in net photosynthetic rate (Pn) after 5, 10, and 15 days of stress are shown in Figure 1 [Figure 1: see original paper]. After 5 days, Pn showed no significant change with increasing salt concentration in Group A (F=0.283, $P>0.05$), an initial increase then decrease in Group B, and a significant decreasing trend in Group C (F=20.415, $P<0.01$) (Figure 1:a). After 10 days, all groups showed significant decreasing trends, particularly Groups A and C (F=17.697, $P<0.01$; F=14.216, $P<0.01$) (Figure 1:b). After 15 days, all groups exhibited extremely significant decreasing trends (F=10.646, $P<0.01$; F=30.713, $P<0.01$; F=19.266, $P<0.01$) (Figure 1:c). In the Qingtongxia simulation group (B2), Pn was significantly lower than B0 and B1 after 5 days, increased after 10 days to levels comparable with B1, then decreased significantly again after 15 days due to prolonged stress.

2.4 Effects of Different Saline-Alkali Stress Treatments on Stomatal Conductance

Stomatal conductance (Gs) showed varying trends with extended stress duration. After 5 days, Group A showed an initial increase then decrease, Group B exhibited irregular fluctuations, and Group C demonstrated a significant decreasing trend with C0 significantly higher than all salt-treated groups (F=23.777, P<0.01) (Figure 1:d). After 10 days, Groups A and B both peaked at A2 and B2 respectively, while Group C (except C0) showed significant decreases with increasing salt concentration (F=15.486, P<0.01) (Figure 1:e). After 15 days, all groups decreased significantly (F=259.488, P<0.01; F=8.718, P<0.01; F=54.844, P<0.01) (Figure 1:f). The Qingtongxia simulation group (B2) showed higher Gs than B0 and B1 after 5 days, significantly higher Gs than all Group B treatments after 10 days, but reduced Gs significantly below B0 after 15 days.

2.5 Effects of Different Saline-Alkali Stress Treatments on Intercellular CO Concentration

Intercellular CO concentration (Ci) fluctuated minimally throughout the stress period, generally following stomatal conductance trends. After 5 days, Group A showed a significant increasing trend, Group B exhibited irregular fluctuations, and Group C decreased significantly with increasing salt concentration (F=11.632, P<0.01) (Figure 2 [Figure 2: see original paper]:a). After 10 days, Groups A and B showed initial increases then decreases, while Group C showed minor fluctuations in C0-C3 but significant decreases in C4 and C5 (Figure 2:b). After 15 days, all groups showed decreasing trends, with significant reductions in Groups A and C (F=54.844, P<0.01; F=22.707, P<0.01) (Figure 2:c). The Qingtongxia simulation group (B2) showed higher Ci than B0 and B1 after 5 days, significantly higher Ci than all Group B treatments after 10 days, and a slight decrease after 15 days.

2.6 Effects of Different Saline-Alkali Stress Treatments on Transpiration Rate

Transpiration rate (Tr) decreased significantly with prolonged saline-alkali stress. No clear patterns were observed in any group after 5 days (Figure 2:d). After 10 days, Groups A and B showed no significant patterns, while Group C decreased significantly with increasing salt concentration (F=26.343, P<0.01) (Figure 2:e). After 15 days, all groups showed significant decreasing trends with increasing salt concentration (F=83.058, P<0.01; F=11.947, P<0.01; F=144.111, P<0.01) (Figure 2:f). In the Qingtongxia simulation group (B2), Tr generally decreased with extended stress duration, showing no significant difference after 5 days (though highest), slightly lower than B0 and B1 after 10 days, and significantly lower than B0 after 15 days.

2.7 Effects of Different Saline-Alkali Stress Treatments on Chlorophyll Fluorescence Parameters Fv/Fm and Fv/Fo

Maximum photochemical efficiency of PSII (Fv/Fm) decreased slightly with prolonged stress but without significant differences among treatments. After 5 days, no significant changes were observed in any group (F=0.265, P>0.05; F=0.148, P>0.05; F=1.056, P>0.05) (Figure 3 [Figure 3: see original paper]:a). After 10 days, Groups A and B showed initial increases then decreases, while Group C decreased with increasing salt concentration, though non-significantly (F=0.649, P>0.05) (Figure 3:b). After 15 days, all groups decreased but without significance (F=1.423, P>0.05; F=0.361, P>0.05; F=0.916, P>0.05) (Figure 3:c). Potential photochemical activity (Fv/Fo) showed similar patterns to Fv/Fm, decreasing over time but without significant differences within groups. In the Qingtongxia simulation group (B2), Fv/Fm and Fv/Fo showed no significant changes after 5 days, reached maximum values at B2 after 10 days, and were slightly lower than B0 and B1 after 15 days without significant differences (Figure 3:d-f).

Discussion and Conclusion

Soil salinization is a major environmental stress factor affecting plant growth and reducing productivity; maintaining above- and below-ground biomass and normal photosynthetic growth under saline-alkali stress is crucial for vegetation improvement in saline regions. Plant height change is the most direct indicator of saline-alkali tolerance (Liu et al., 2015). This study demonstrated that plant height increments were higher under low (0, 0.2%) and moderate (0.4%, 0.6%) salt concentrations than under high (0.8%, 1.0%) concentrations. Compared to pH=7.0 and pH=8.0 treatments, pH=9.0 treatments showed height decline earlier (after 10 days), indicating that salt concentration has greater impact under mild alkali stress, while alkali stress dominates under severe conditions, with alkali effects exceeding salt effects. Roots, being the organs in direct and prolonged contact with saline-alkali solutions, more directly reflect plant growth status than stems and leaves (Lin et al., 2011). Results showed that pH=7.0 and pH=8.0 treatments promoted greater root elongation, while pH=9.0 treatments produced the least and non-significant root growth. Within pH=8.0 treatments, low salt concentrations (0, 0.2%) significantly promoted root elongation, while moderate (0.4%, 0.6%) and high (0.8%, 1.0%) concentrations showed minimal changes. Zhao (2010) reported that root growth is optimal at pH 7.0-8.0, as roots release small organic acids that regulate rhizosphere pH to optimal levels; when soil pH exceeds the adjustable range, root growth becomes inhibited. In the Qingtongxia simulation group (B2), both plant height and root length maintained growth with an overall pattern of initial reduction followed by increased growth rate, indicating that 'Hanluhong' can gradually adapt to Qingtongxia' s saline-alkali stress through self-regulation. This may occur because saline-alkali stress promotes reallocation of carbon assimilation products, increasing the proportion of dry matter distributed to roots relative to above-ground parts, thereby enlarging root systems, increasing absorption area, and providing adequate nutrients to support plant growth (Xia et al., 2015).

Photosynthetic capacity critically influences plant development and stress resistance, making photosynthetic parameters valuable indicators for assessing plant growth and stress tolerance (Wang et al., 2011). This study revealed that prolonged saline-alkali stress decreased net photosynthetic rate, stomatal conductance, and intercellular CO concentration across all pH gradients. In the Qingtongxia simulation group (B2), although net photosynthetic rate, stomatal conductance, and transpiration rate were slightly lower than controls, intercellular CO concentration showed no significant difference from controls. This pattern likely results from stomatal effects (Zheng et al., 2002), where saline-alkali stress induces K efflux from roots, creating ionic imbalance that reduces stomatal conductance and photosynthesis, consequently decreasing CO consumption.

Excitation energy capture and conversion are coordinated by photosystems I and II; imbalance between these processes disrupts electron transport or excitation status. When absorbed light energy exceeds utilization capacity, excess energy causes photoinhibition and even photodamage, with decreased Fv/Fm being the most obvious characteristic of photoinhibition (Everard et al., 1994; Lu et al., 2003). In this study, Fv/Fm and Fv/Fo decreased slightly after saline-alkali stress but not significantly, indicating that while stress reduced photosynthetic organ activity and carbon fixation, it did not cause severe blockage of photosynthetic electron transport or photoinhibition, and the photosynthetic system was not seriously damaged. After 10 days of stress, Fv/Fo and Fv/Fm in pH=8.0 treatments increased initially then decreased, peaking in the Qingtongxia simulation group (B2). Correspondingly, stomatal conductance and intercellular CO concentration also reached maximum values, while net photosynthetic rate and transpiration rate increased compared to day 5, suggesting that moderate saline-alkali concentrations temporarily enhanced photosynthesis, improving electron acceptance and transfer capacity and consequently increasing chlorophyll fluorescence parameters. After 15 days, the Qingtongxia simulation group showed slight but non-significant decreases in Fv/Fm and Fv/Fo, indicating that photosynthetic electron transport was not severely affected and the plants maintained a certain level of saline-alkali tolerance.

Mixed saline-alkali stress affected plant height, root length, photosynthetic characteristics, and chlorophyll fluorescence to varying degrees. Prolonged high-salt or high-alkali environments were detrimental to 'Hanluhong' growth and photosynthesis. However, regarding adaptability in Qingtongxia, the plants maintained normal photosynthesis and above- and below-ground growth under moderate saline-alkali stress conditions. Although PSII potential photochemical activity and maximum photochemical efficiency decreased slightly, the reductions were not significant, indicating essentially normal photosynthetic electron transport and that saline-alkali stress remained within the plant' s adjustable range. Therefore, ground-cover chrysanthemum 'Hanluhong' possesses certain saline-alkali tolerance and can basically grow normally under Qingtongxia' s saline-alkali stress intensity, making it suitable for vegetation and ecological environment restoration.

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