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NO EVIDENCE FOR DIFFERENTIAL BIOMASS AND MINERAL CONTENT IN ADULT PLANTS GROWN FROM DIMORPHIC *SUAEDA ARALOCASPICA* SEEDS

LEI WANG[1], HONG-LING WANG[1], CHANG-YAN TIAN[1*] AND ZHEN-YING HUANG[2]

[1]State Key Laboratory of Desert and Oasis Ecology, Xinjiang Institute of Ecology and Geography,
Chinese Academy of Sciences, Urumqi 830011, China
[2]State Key Laboratory of Vegetation and Environmental Change, Institute of Botany,
Chinese Academy of Sciences, Beijing 100093, China
**Corresponding author's e-mail: halophyte@gmail.com; Tel: +86-991-7885301; Fax: +86-991-7885320.*

Abstract

The production of two or more seed types by a single plant is known as seed heteromorphism. There have been many comparisons of seed traits or growth between plants grown from heteromorphic seeds. However, information is scarce regarding the mineral contents of adult plants from heteromorphic seeds. We herein present biomass and mineral profiles of adult plants grown from dimorphic seeds (non-dormant brown seeds and black seeds with non-deep physiological dormancy) of the annual desert halophyte *Suaeda aralocaspica* at different nutrient and salinity levels. Although nutrient and salinity treatments affected dry weight and mineral content, there were no significant differences among *S. aralocaspica* seed-dimorphic plants under the same experimental conditions. This study is one of the few to compare the physiological responses between seed-heteromorphic plants, and reveals that mineral status corresponds with growth performance in these plants.

Key words: Dimorphic, *Suaeda aralocaspica*, Heteromorphic, Biomass

Introduction

Seed heteromorphism refers to the production of different seed types from an individual plant. Heteromorphic seeds usually differ in their ecological and biological characteristics, such as color and shape (Brändel, 2007; Song *et al.*, 2008; Ei-Keblawy *et al.*, 2014), germination (Khan *et al.*, 2001; Yao *et al.*, 2010; Aguado *et al.*, 2011), dispersability (Talavera *et al.*, 2010), and storage (i.e., soil seed bank) (Mandák & Pyšek ,2001; Cao *et al.*, 2012). For example, differences between peripheral and central achenes of the annual heterocarpic species *Leontodon longirrostris* result in differences in dispersal and germination (Ruiz de Clavijo, 2001).

There are numerous studies on seed-heteromorphic plants that describe the carry-over of differential growth and reproduction characteristics, including seedling establishment (Venable & Levin, 1985), plant size (Cheplick & Quinn, 1982), salt tolerance (Redondo-Gómez *et al.*, 2008; Jiang *et al.*, 2012), phenotypic plasticity (Zhang, 1995), competitive ability (Imbert *et al.*, 1997), and reproductive allocation (Sadeh *et al.*, 2009; Lu *et al.*, 2013). Plants grown from heteromorphic seeds generally show different growth characteristics under competitive conditions. However, in some cases, such differences disappear in the later stages of the life cycle. For example, *Emex spinosa* plants grown from subterranean seeds have larger leaf areas and stems than plants grown from aerial achenes in mixed cultures, but these differences do not exist in monocultures (Weiss, 1980).

Growth indices (e.g., biomass and plant height) have been used to compare seed-heteromorphic plants (Cheplick & Quinn, 1982; Ruiz de Clavijo & Jiménez, 1998; Ruiz de Clavijo, 2001; Mandák & Pyšek, 2005), and a few studies have compared the physiological responses (antioxidative enzyme activities, chlorophyll content, chlorophyll fluorescence, gas exchange and ion accumulation) between seedlings and adult plants grown from heteromorphic seeds (Redondo-Gómez *et al.*, 2008; Xu *et al.*, 2011; Jiang *et al.*, 2012). These studies revealed that plants grown from different seed types exhibit differences in physiological responses. Additionally, there is no report of the absence of physiological differences among plants grown from heteromorphic seeds.

Suaeda aralocaspica (Bunge) Freitag & Schütze (Amaranthaceae) is an annual halophyte restricted to the saline-alkaline soils of the Gobi desert in central Asia. It germinates from late March to April, and flowering occurs from late August to September, with fruits maturing and dispersing in October (Wang *et al.*, 2012). This species produces two seed types from a single plant. Brown seeds are oblate, non-dormant, and can germinate rapidly under relatively high salinity conditions. Black seeds are biconvex, have type-2 non-deep physiological dormancy, and germinate slowly even under low salinity conditions (Wang *et al.*, 2008).

In this study, we hypothesized that the mineral content of adult plants grown from heteromorphic seeds corresponded with growth performance. We designed a single-plant pot experiment to compare the biomass and mineral profiles (Cl, Na, N, P, and K) of adult plants grown from dimorphic seeds of *S. aralocaspica* under different nutrient and salinity levels.

Materials and Methods

Seed collection: In early October 2008, freshly matured *S. aralocaspica* fruits were collected from a natural population of approximately 600 individuals (44°14′N, 87°44′E; 445 m a.s.l.) in a saline desert in the northern part of Xinjiang Province, China [for a detailed description of this site, please refer to Wang *et al.* (2012)]. The dry fruits were manually rubbed to detach the seeds enclosed by the membranous pericarps. The brown and black seeds were stored separately at 4°C.

Experimental design: The experiment was completed in a screen house covered with a sun shade net (approximately 25% porosity) at the Fukang Desert Ecosystem Observation and Experimental Station of the Chinese Academy of Sciences (44º17′26″N, 87º55′58″E; 460 m a.s.l.). We used a randomized block design with six replicates. Each block consisted of 18 pots representing two seed types (brown and black), three fertilization treatments (low, moderate, and high), and three salinity treatments [none (control), low, and high].

Fertilization treatments involved Osmocote 301 granular lawn fertilizer (15 N: 11 P: 13 K: 2 Mg; Scotts, USA) as the basic fertilizer. Peters1 commercial fertilizer (20 N: 20 P: 20 K; Scotts, USA) was used as a source of supplemental nutrients. For low, moderate, and high fertilization levels, plants were treated once a week with 1.2 g Osmocote 301 and 100 mL Peters1 nutrient solution (0.046 g L^{-1}), 6 g Osmocote 301 and 100 mL Peters1 nutrient solution (0.23 g L^{-1}), or 12 g Osmocote 301 and 100 mL Peters1 nutrient solution (0.46 g L^{-1}), respectively. The Peters1 nutrient solution was added starting 3 weeks after sowing.

Salinity treatments involved a mixed salt solution consisting of NaCl: Na_2SO_4: $NaHCO_3$ (20:20:1 mass ratio). For low and high salinity levels, plants were treated once a week with 1 g L^{-1} salt solution (100 mL) or 7 g L^{-1} salt solution (100 ml), respectively. Control plants were treated with 100 mL tap water. To avoid salt shock, the 7 g L^{-1} salt solution was gradually applied by increasing the salt concentration by 1 g L^{-1} each week. Salinity treatments started 4 weeks after planting.

Approximately 20 brown or black seeds were sown in plastic pots (17-cm deep and 16-cm in diameter) filled with 2000 cm^3 vermiculite quartz: sand mix (4:1, v/v) in late April 2009. Germinated seedlings were grown for nearly 3 weeks and then thinned to one seedling per pot. To reduce the variability of initial seedling size, only seedlings of similar height were used for each seed type. Pots were watered every 2 d, and the same amount of water/nutrient solution/salt solution was used for each pot. Plants were harvested 23 weeks after sowing.

Measurements and chemical analyses: Shoot samples were oven-dried to a constant weight at 75°C for 2 d, then weighed using an electronic analytical balance (accuracy: 0.01 g). The shoot samples were ground and stored in plastic bags. A water extract method was used to extract Na and Cl. Flame photometric and titrimetric methods were used to measure Na and Cl contents, respectively. The total N, P, and K contents were determined using 0.5 g samples, which were transferred to 100-ml digestion flasks containing 5 ml concentrated H_2SO_4. The samples were then treated twice with 4 ml H_2O_2. The flasks were heated at 360°C for 10 min, after which 2 ml H_2O_2 was added. This step was repeated until the digested samples became colorless. Flame photometric, vanadium molybdate yellow colorimetric, and distillation methods were used to measure K, P, and N contents, respectively.

Statistical analysis: A three-way analysis of variance was used to determine the effects of plant type, salinity, and nutrient levels as well as their interactions on dry matter yields and mineral concentrations. The Tukey test was completed for multiple comparisons to determine significant ($p<0.05$) differences between individual treatments. All data were analyzed using SPSS, version 16.0 (SPSS Inc., Chicago, IL, USA).

Results

The plant type had no significant effect on biomass ($p=0.634$). Additionally, there were no significant interactive effects among all three factors examined. In contrast, salinity or nutrient levels significantly affected plant biomass (Table 1). Salinity increased plant biomass at moderate and high nutrient levels, but not at low nutrient levels. Nutrient supplementation also increased plant biomass. The highest biomass was observed at high salinity and high nutrient levels (Fig. 1).

The main effects of plant type on Na and Cl concentrations were not significant (Table 1). However, the main effects of salinity on Na and Cl concentrations were significant, with salinity increasing Cl concentrations at all nutrient levels. In contrast, nutrient supplementation decreased Cl concentrations. The highest Cl concentrations were observed at high salinity and low nutrient levels. The highest Na concentrations occurred under high salinity and moderate or high nutrient levels (Fig. 2). There were significant plant type × salinity and plant type × nutrient effects on Na concentration, and significant salinity × nutrient effects on Cl concentration.

Table 1. Three-way analysis of variance of the effects of plant type, salinity, and nutrient levels as well as their interactions on *Suaeda aralocaspica* biomass and mineral contents (Cl, Na, N, P, and K).

Source	Biomass		Cl		Na		N		P		K	
	***F*-value**	***P*-value**	***F*-value**	***P*-value**	***F*-value**	***P*-value**	***F*-value**	***P*-value**	***F*-value**	***P*-value**	***F*-value**	***P*-value**
Plant type (PT)	0.04	0.947	0.05	0.825	1.74	0.193	0.741	0.393	0.387	0.536	2.565	0.115
Salt (S)	13.248	<0.001	461.22	<0.001	9.911	<0.001	28.763	<0.001	40.287	<0.001	0.828	0.442
Nutrient (N)	336.666	<0.001	111.618	<0.001	33.885	<0.001	18.992	<0.001	3.209	0.048	11.567	<0.001
PT*S	0.415	0.661	0.293	0.747	9.294	<0.001	1.335	0.272	3.833	0.028	3.035	0.056
PT*N	0.609	0.546	0.883	0.419	3.264	0.046	2.974	0.06	1.275	0.288	6.824	0.002
S*N	1.538	0.198	9.496	<0.001	1.51	0.212	3.474	0.013	7.805	<0.001	6.529	<0.001
PT*S*N	2.347	0.06	0.427	0.788	3.271	0.018	0.612	0.656	2.049	0.1	0.959	0.438

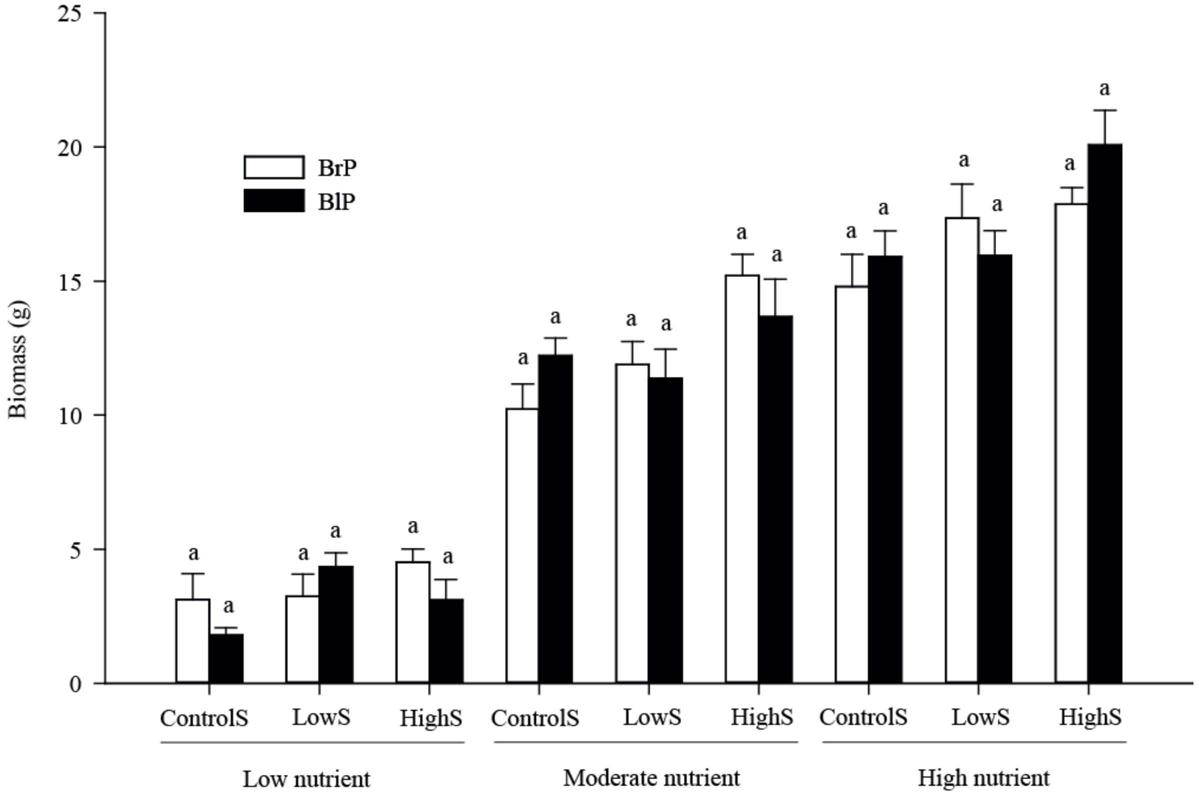

Fig. 1. Effects of plant type, salinity and nutrient levels on biomass. BrP: plant grown from brown seed; BlP: Plant grown from black seed; ControlS: Control salinity; LowS: Low salinity; HighS: High salinity. Bars with the same letters indicate no significant differences in dry weight of seed-dimorphic plants at a given salinity under a particular nutrient treatment at $p<0.05$ level.

The main effects of plant type on N ($p=0.393$), P ($p=0.536$), and K ($p=0.115$) concentrations were not significant. However, the main effects of salinity on N and P concentrations, and the main effects of nutrients on N, P, and K concentrations were significant (Table 1). There were significant plant type × salinity and plant type × nutrient effects on P and K concentrations, respectively. Additionally, there was a significant salinity × nutrient effect on N, P, and K concentrations. Overall, there were no significant differences in N, P, and K concentrations between plants from brown or black seeds. Salinity significantly decreased the N and P concentrations at moderate and high nutrient levels, but not at low nutrient levels (Fig. 3).

Discussion

Although differences among plants grown from heteromorphic seeds have been studied extensively (for reviews see Mandák, 1997 and Imbert, 2002), the current study is the first in which no significant differences in mineral contents were observed among adult plants grown from heteromorphic seeds. Dimorphic *S. aralocaspica* seeds have variable mass, dormancy, and germination characteristics (Wang *et al.*, 2008). However, there are no differences in adult seed-dimorphic plants in terms of biomass and mineral content. Therefore, the results of this study support our hypothesis that mineral status corresponds with growth performance in plants grown from heteromorphic seeds.

It is often hypothesized that plants grown from heteromorphic seeds differ in competitive ability and stress tolerance (Mandák, 1997; Redondo-Gómez *et al.*, 2008). Some seed-heteromorphic species can maintain carry-over differences throughout their life cycle, even in the absence of stress (Weiss, 1980; Venable & Levin, 1985; Mandák & Pyšek, 2005). In contrast, the biomass of reproductive tissues of the seed-heteromorphic *Crepis sancta* remains unchanged, even in the absence of competition (Imbert *et al.*, 1997). Additionally, there are no productivity differences between mixed cultures and monocultures of *Dimorphotheca sinuata* (Beneke *et al.*, 1992). There are no apparent fixed patterns in seed-heteromorphic plants.

Seed-heteromorphic plant growth may be related to physiological factors. Seedlings grown from brown and black *Suaeda splendens* seeds grow well at 400 mmol L^{-1} NaCl, but seedlings grown from brown seeds grow poorly under low salinity conditions. Growth differences among seed-dimorphic plants may be associated with chlorophyll fluorescence and gas exchange (Redondo-Gómez *et al.*, 2008). Jiang *et al.* (2012) reported that the growth status of *Suaeda salsa* adult plants from dimorphic seeds was related to chlorophyll content and photosynthetic rate. Seedlings grown from yellow seeds of *Atriplex centralasiatica*, which is highly tolerant to salinity stress, produce greater amounts of NO than seedlings from brown seeds (Xu *et al.*, 2011). It is clear that physiological differences can reflect growth differences in seed-heteromorphic plants. Furthermore, our results reveal that similar mineral contents will result in similar growth performances among seed-heteromorphic plants.

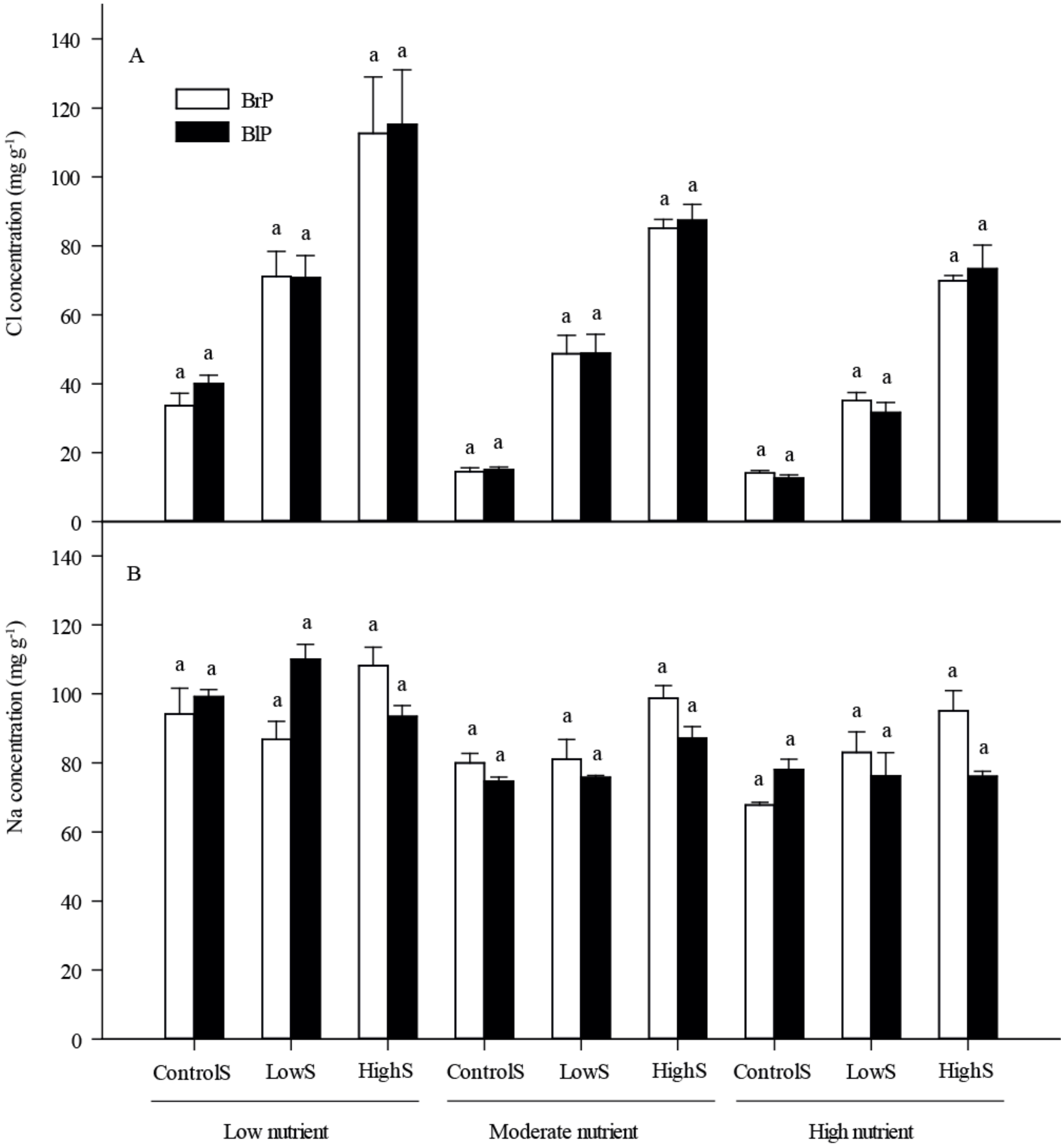

Fig. 2. Effects of plant type, salinity and nutrient levels on Cl and Na concentration. BrP: plant grown from brown seed; BlP: plant grown from black seed; ControlS: Control salinity; LowS: Low salinity; HighS: High salinity. Bars with the same letters indicate no significant differences in Cl or Na concentration of seed-dimorphic plants at a given salinity under a particular nutrient treatment at $P < 0.05$ level.

Plants grown from the two *S. aralocaspica* seed types analyzed in this study exhibited similar responses to salinity and nutrient availability. However, mineral content is more sensitive to environmental factors than biomass. This was confirmed by the significant effects between plant type and salinity or plant type and nutrients on different mineral concentrations. At salinity and nutrient levels beyond those used in this study, the differences between plants grown from dimorphic seeds will be observed first in the mineral concentrations.

In conclusion, we confirmed that adult plants grown from dimorphic *S. aralocaspica* seeds were similar in terms of biomass and mineral content under the same environmental conditions. Further studies are necessary to clarify whether the absence of differences in heteromorphic plants is related to other environmental constraints.

Acknowledgements

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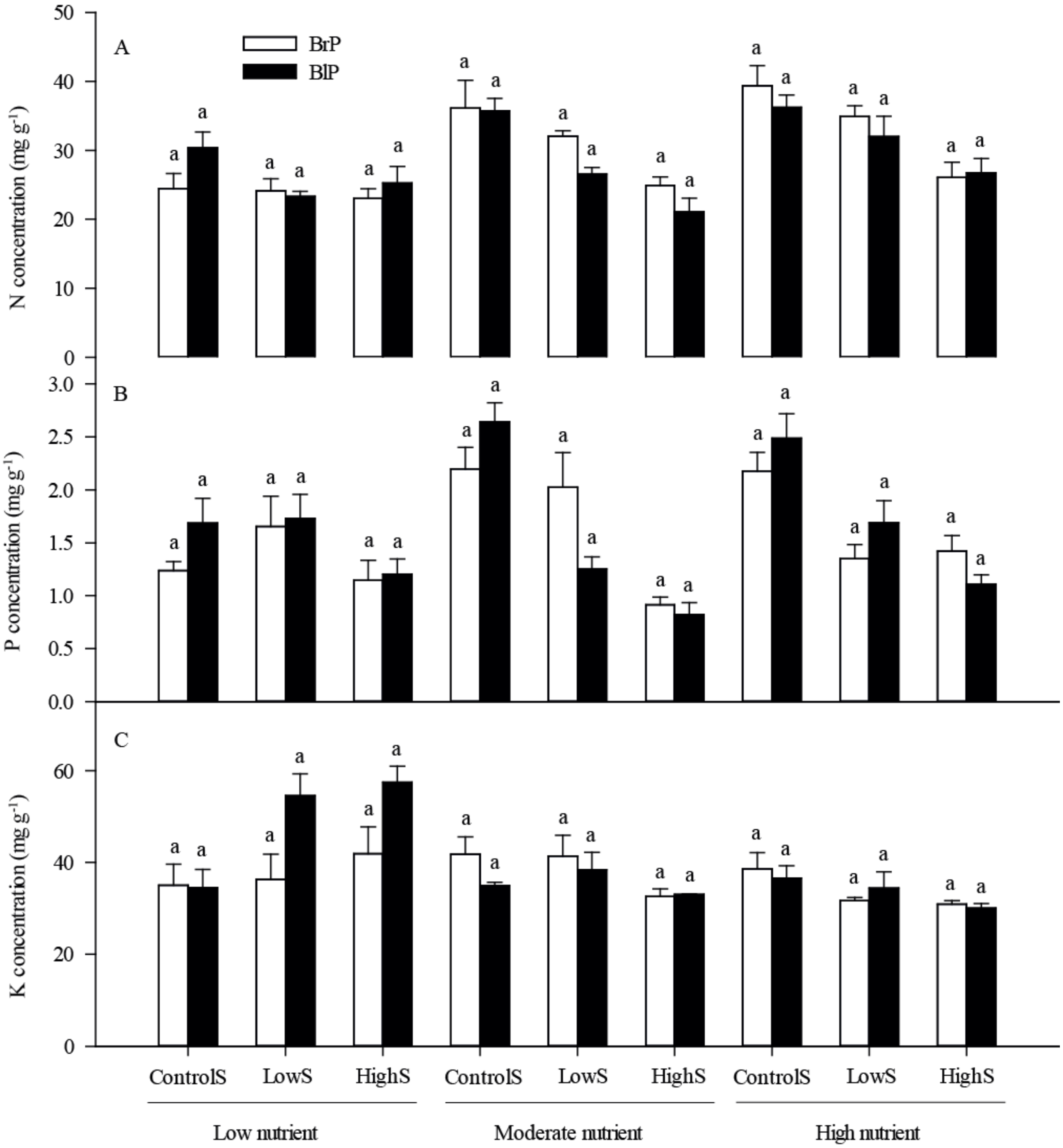

Fig. 3. Effects of plant type, salinity and nutrient levels on N, P and K concentration. BrP: plant grown from brown seed; BlP: plant grown from black seed; ControlS: Control salinity; LowS: Low salinity; HighS: High salinity. Bars with the same letters indicate no significant differences in N, P or K concentration concentration of seed-dimorphic plants at a given salinity under a particular nutrient treatment at $p<0.05$ level.

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